MAGEC2 IMMUNOGENIC PEPTIDES, BINDING PROTEINS RECOGNIZING MAGEC2 IMMUNOGENIC PEPTIDES, AND USES THEREOF

Cross-Reference to Related Application

This application claims the benefit of priority to U.S. Provisional Application Serial No. 63/631,364, filed on 08 April 2024; the entire contents of said application are incorporated herein in their entirety by this reference.

Background of the Invention

Adoptive cell transfer (ACT) using engineered T cells has demonstrated great efficacy in treating certain types of liquid tumor and holds promise for treating solid tumor. T cell receptor-engineered T cells (TCR-T) are T cells expressing an exogenous TCR that recognizes an antigen that exist in cancer cells. The TCR-antigen interaction is the core component of the targeting mechanism that allows the TCR-T cells to kill cancer cells. One of the challenges for the broad testing and adoption of TCR-T therapy is the lack of TCR-antigen pairs that are applicable to a wide range of patients and indications. Although several TCR and antigens have been explored in clinical trials, most pursued antigens are restricted to one HLA allele, namely HLA-A*02:01, which limits the number of patients who are eligible for the therapy and also allowing cancer cells to develop potential resistance by mutating the HLA-A*02:01 allele.

In addition, the number of pursued antigens is limited due to the difficulty of discovering novel TCR-antigen pairs which commonly require prediction of the MHC presented epitope. However, such epitopes may not be immunogenic, thereby making it difficult to identify a reactive TCR, or the epitope may not be processed and presented physiologically by the cancer cells. Accordingly, there is a great need in the art to identify TCR-antigen pairs in the context of a variety of widely applicable HLA alleles in order to develop useful reagents to diagnose, prognose, treat, and screen agents relevant for disorders characterized by the expression of the antigens.

Summary of the Invention

The present invention is based, at least in part, on the discovery of MAGEC2 immunogenic peptides and binding proteins recognizing such MAGEC2 immunogenic peptides based on unbiased functional screens used to discover the antigen of TCR clonotypes identified from subjects having disorders associated with MAGEC2 expression

(*e.g.*, subjects afflicted with as melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, or bladder urothelial carcinoma). The identified TCRs recognized MAGEC2 immunogenic peptides, such as those listed in Table 1, in the context of a variety of HLA alleles (*e.g.*, HLA-B*07:02). MAGEC2 is demonstrated herein to be selectively expressed in cancer and testis tissue, but not in normal somatic tissues, thereby making it an ideal target for ACT. The ability of MAGEC2 binding proteins (*e.g.*, TCRs described herein) to bind MAGEC2 immunogenic peptides and to elicit immune responses that kill cells expressing MAGEC2 (*e.g.*, cancer cells) demonstrates the utility of such binding proteins in a diversity of uses, including methods of diagnosis, prognosis, treatment, and screening of agents relevant for disorders characterized by MAGEC2 expression.

Efficient screening and optimization of TCR sequences stands as a necessary step towards identifying optimal functionality. TCRs provided herein were identified from a large-scale screen of 25,000 TCRs from the naïve T cell repertoire of donor blood, then 200 TCR sequences were optimized from their original form to efficiently express the TCR sequences in donor cells for adoptive cell therapy (ACT). To select TCRs with the optimal function of the codon-optimized TCR sequences, each TCR was evaluated for cell killing, cytokine release, and proliferation upon co-culture with cancer cell models expressing MAGEC2 both *in vitro* and in mouse models. Off-target reactivity and off-target HLA alloreactivity of TCRs pose a significant safety concern for ACT. Therefore, the TCRs herein were evaluated using a genome-wide off-target screen, and selected based on specific off-target profiles as well as alloreactivity profiles that are conducive for a safe and effective ACT product.

In one aspect, an immunogenic peptide comprising a peptide epitope selected from peptide sequences listed in Table 1, is provided.

In another aspect, an immunogenic peptide consisting of a peptide epitope selected from peptide sequences listed in Table 1, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, the immunogenic peptide is derived from a MAGEC2 protein, optionally wherein the immunogenic peptide is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length. In another embodiment, the immunogenic peptide is capable of eliciting an immune response against MAGEC2 and/or MAGEC2-expressing cells in a subject, optionally wherein the immune response is i) a T cell

response and/or a CD8+ T cell response and/or ii) selected from the group consisting of T cell expansion (*e.g.*, proliferation), cytokine release, and/or cytotoxic killing.

In still another aspect, an immunogenic composition comprising at least one immunogenic peptide described herein, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, the immunogenic composition further comprises an adjuvant. In another embodiment, the immunogenic composition is capable of eliciting an immune response against MAGEC2 and/or MAGEC2-expressing cells in a subject, optionally wherein the immune response is i) a T cell response and/or a CD8+ T cell response and/or ii) selected from the group consisting of T cell expansion (*e.g.*, proliferation), cytokine release, and/or cytotoxic killing.

In yet another aspect, a composition comprising a peptide epitope selected from peptide sequences listed in Table 1, and an MHC molecule, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, the MHC molecule is an MHC multimer, optionally wherein the MHC multimer is a tetramer. In another embodiment, the MHC molecule is an MHC class I molecule. In still another embodiment, the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702.

In another aspect, a stable MHC-peptide complex, comprising an immunogenic peptide described herein in the context of an MHC molecule, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, the MHC molecule is an MHC multimer, optionally wherein the MHC multimer is a tetramer. In another embodiment, the MHC molecule is an MHC class I molecule. In still another embodiment, the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702. In yet another embodiment, the peptide epitope and the MHC molecule are covalently linked and/or wherein the alpha and beta chains of the MHC molecule are covalently linked. In another embodiment, the stable MHC-peptide complex comprises a detectable label, optionally wherein the detectable label is a fluorophore.

In still another aspect, an immunogenic composition comprising a stable MHC-peptide complex described herein, and an adjuvant, is provided.

In yet another aspect, an isolated nucleic acid that encodes an immunogenic peptide described herein, or a complement thereof, is provided.

In another aspect, a vector comprising an isolated nucleic acid described herein, is provided.

In still another aspect, a cell that a) comprises an isolated nucleic acid described herein, b) comprises a vector described herein, and/or c) produces one or more immunogenic peptides described herein and/or presents at the cell surface one or more stable MHC-peptide complexes described herein, optionally wherein the cell is genetically engineered, is provided.

In yet another aspect, a device or kit comprising a) one or more immunogenic peptides described herein and/or b) one or more stable MHC-peptide complexes described herein, said device or kit optionally comprising a reagent to detect binding of a) and/or b) to a binding protein, optionally wherein the binding protein is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain, is provided.

In another aspect, a method of detecting T cells that bind a stable MHC-peptide complex comprising: a) contacting a sample comprising T cells with a stable MHC-peptide complex described herein; and b) detecting binding of T cells to the stable MHC-peptide complex, optionally further determining the percentage of stable MHC-peptide-specific T cells that bind to the stable MHC-peptide complex, optionally wherein the sample comprises peripheral blood mononuclear cells (PBMCs), is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, T cells are CD8+ T cells. In another embodiment, detecting and/or determining is performed using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay. In still another embodiment, a sample comprises T cells contacted with, or suspected of having been contacted with, one or more MAGEC2 proteins or fragments thereof.

In still another aspect, a method of determining whether a T cell has had exposure to MAGEC2 comprising: a) incubating a cell population comprising T cells with an

immunogenic peptide described herein or a stable MHC-peptide complex described herein; and b) detecting the presence or level of reactivity, wherein the presence of or a higher level of reactivity compared to a control level indicates that the T cell has had exposure to MAGEC2, optionally wherein the cell population comprising T cells is obtained from a subject, is provided.

In yet another aspect, a method for predicting the clinical outcome of a subject afflicted with a disorder characterized by MAGEC2 expression comprising: a) determining the presence or level of reactivity between T cells obtained from the subject and one more immunogenic peptides described herein or one or more stable MHC-peptide complexes described herein; and b) comparing the presence or level of reactivity to that from a control, wherein the control is obtained from a subject having a good clinical outcome, wherein the presence or a higher level of reactivity in the subject sample as compared to the control indicates that the subject has a good clinical outcome, is provided.

In another aspect, a method of assessing the efficacy of a therapy for a disorder characterized by MAGEC2 expression comprising: a) determining the presence or level of reactivity between T cells obtained from the subject and one more immunogenic peptides described herein or one or more stable MHC-peptide complexes described herein, in a first sample obtained from the subject prior to providing at least a portion of the therapy to the subject, and b) determining the presence or level of reactivity between the one more immunogenic peptides described herein, or the one or more stable MHC-peptide complexes described herein, and T cells obtained from the subject present in a second sample obtained from the subject following provision of the therapy to the subject, wherein the presence or a higher level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the disorder characterized by MAGEC2 expression in the subject, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, the level of reactivity is indicated by a) the presence of binding and/or b) T cell activation and/or effector function, optionally wherein the T cell activation or effector function is T cell proliferation, killing, or cytokine release. In another embodiment, a method further comprises repeating steps a) and b) at a subsequent point in time, optionally wherein the subject has undergone treatment to ameliorate the disorder characterized by MAGEC2 expression between the first point in time and the subsequent point in time. In still another embodiment, T cell binding, activation, and/or

effector function is detected using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay. In yet another embodiment, a control level is a reference number. In another embodiment, a control level is a level of a subject without the disorder characterized by MAGEC2 expression.

In still another aspect, a method of preventing and/or treating a disorder characterized by MAGEC2 expression in a subject comprising administering to the subject a therapeutically effective amount of a composition described herein.

In yet another aspect, a method of identifying a peptide-binding molecule, or antigen-binding fragment thereof, that binds to a peptide epitope selected from the peptide sequences listed in Table 1 comprising: a) providing a cell presenting a peptide epitope selected from the peptide sequences listed in Table 1 in the context of an MHC molecule on the surface of the cell; b) determining binding of a plurality of candidate peptide-binding molecules or antigen-binding fragments thereof to the peptide epitope in the context of the MHC molecule on the cell; and c) identifying one or more peptide-binding molecules or antigen-binding fragments thereof that bind to the peptide epitope in the context of the MHC molecule, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a step a) comprises contacting the MHC molecule on the surface of the cell with a peptide epitope selected from the peptide sequences listed in Table 1. In another embodiment, a step a) comprises expressing the peptide epitope selected from the peptide sequences listed in Table 1 in the cell using a vector comprising a heterologous sequence encoding the peptide epitope.

In another aspect, a method of identifying a peptide-binding molecule or antigen-binding fragment thereof that binds to a peptide epitope selected from the peptide sequences listed in Table 1 comprising: a) providing a peptide epitope either alone or in a stable MHC-peptide complex, comprising a peptide epitope selected from the peptide sequences listed in Table 1, either alone or in the context of an MHC molecule; b) determining binding of a plurality of candidate peptide-binding molecules or antigen-binding fragments thereof to the peptide or stable MHC-peptide complex; and c) identifying one or more peptide-binding molecules or antigen-binding fragments thereof that bind to the peptide epitope or the stable MHC-peptide complex, optionally wherein the MHC or MHC-peptide complex is as described herein, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a plurality of candidate peptide binding molecules comprises an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain. In another embodiment, a plurality of candidate peptide binding molecules comprises at least 2, 5, 10, 100, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or more, different candidate peptide binding molecules. In still another embodiment, a plurality of candidate peptide binding molecules comprises one or more candidate peptide binding molecules that are obtained from a sample from a subject or a population of subjects; or the plurality of candidate peptide binding molecules comprises one or more candidate peptide binding molecules that comprise mutations in a parent scaffold peptide binding molecule obtained from a sample from a subject. In yet another embodiment, a subject or population of subjects are a) not afflicted with a disorder characterized by MAGEC2 expression and/or have recovered from a disorder characterized by MAGEC2 expression, or b) are afllicted with a disorder characterized by MAGEC2 expression. In another embodiment, a subject or population of subjects has been administered a composition described herein. In still another embodiment, a subject is an animal model of a disorder characterized by MAGEC2 expression and/or a mammal, optionally wherein the mammal is a human, a primate, or a rodent. In yet another embodiment, a subject is an animal model of a disorder characterized by MAGEC2 expression, an HLA-transgenic mouse, and/or a human TCR transgenic mouse. In another embodiment, a sample comprises peripheral blood mononuclear cells (PBMCs), T cells, and/or CD8+ memory T cells.

In still another aspect, a peptide-binding molecule or antigen-binding fragment thereof identified according to a method described herein, optionally wherein the peptide-binding molecule or antigen-binding fragment thereof is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain, is provided.

In yet another aspect, a method of treating a disorder characterized by MAGEC2 expression in a subject comprising administering to the subject a therapeutically effective amount of genetically engineered T cells that express a peptide-binding molecule or antigen-binding fragment thereof that i) binds to a peptide epitope selected from the sequences listed in Table 1, ii) is identified according to a method described herein, and/or iii) binds to a

stable MHC-peptide complex comprising a peptide epitopes selected from the sequences listed in Table 1 in the context of an MHC molecule, optionally wherein the peptide-binding molecule or antigen-binding fragment thereof is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain, optionally wherein the MHC or MHC-peptide complex is as described herein, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, T cells are isolated from a) the subject, b) a donor not afflicted with the disorder characterized by MAGEC2 expression, or c) a donor recovered from a disorder characterized by MAGEC2 expression.

In another aspect, a method of treating a disorder characterized by MAGEC2 expression in a subject comprising transfusing antigen-specific T cells to the subject, wherein the antigen-specific T cells are generated by: a) stimulating immune cells from a subject with a composition described herein; and b) expanding antigen-specific T cells *in vitro* or *ex vivo*, optionally i) isolating immune cells from the subject before stimulating the immune cells and/or ii) wherein the immune cells comprise PBMCs, T cells, CD8+ T cells, naive T cells, central memory T cells, and/or effector memory T cells, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, agents are placed in contact under conditions and for a time suitable for the formation of at least one immune complex between the peptide epitope, immunogenic peptide, stable MHC-peptide complex, T cell receptor, and/or immune cells. In another embodiment, a peptide epitope, immunogenic peptide, stable MHC-peptide complex, and/or T cell receptor is expressed by cells and the cells are expanded and/or isolated during one or more steps. In still another embodiment, a disorder characterized by MAGEC2 expression is a cancer or relapse thereof, optionally wherein the cancer is selected from the group consisting of melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma. In yet another embodiment, a subject is an animal model of a disorder characterized by MAGEC2 expression and/or a mammal, optionally wherein the mammal is a human, a primate, or a rodent.

In still another aspect, a binding protein that binds a polypeptide comprising an immunogenic peptide sequence described herein, an immunogenic peptide described herein, and/or the stable MHC-peptide complex described herein, optionally wherein the binding protein is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a binding protein comprises: a) a T cell receptor (TCR) alpha chain CDR sequence with at least about 80% identity to a TCR alpha chain CDR sequence selected from the group consisting of TCR alpha chain CDR sequences listed in Table 2; and/or b) a TCR beta chain CDR sequence with at least about 80% identity to a TCR beta chain CDR sequence selected from the group consisting of TCR beta chain CDR sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M. In another embodiment, a binding protein comprises: a) a TCR alpha chain variable (V_α) domain sequence with at least about 80% identity to a TCR V_α domain sequence selected from the group consisting of TCR V_α domain sequences listed in Table 2; and/or b) a TCR beta chain variable (V_β) domain sequence with at least about 80% identity to a TCR V_β domain sequence selected from the group consisting of TCR V_β domain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M. In still another embodiment, a binding protein comprises: a) a TCR alpha chain sequence with at least about 80% identity to a TCR alpha chain sequence selected from the group consisting of TCR alpha chain sequences listed in Table 2; and/or b) a TCR beta chain sequence with at least about 80% identity to a TCR beta chain sequence selected from the group consisting of TCR beta chain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M. In yet another embodiment, a binding protein comprises: a) a TCR alpha chain CDR sequence selected from the group consisting of TCR alpha chain CDR sequences listed in Table 2; and/or b) a TCR beta chain CDR sequence selected from the group consisting of TCR beta chain CDR sequences listed in Table 2,

wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M. In yet another embodiment, a binding protein comprises: a) a TCR alpha chain variable (V_α) domain sequence selected from the group consisting of TCR V_α domain sequences listed in Table 2; and/or b) a TCR beta chain variable (V_β) domain sequence selected from the group consisting of TCR V_β domain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M, is provided. In another embodiment, a binding protein comprises: a) a TCR alpha chain sequence selected from the group consisting of TCR alpha chain sequences listed in Table 2; and/or b) a TCR beta chain sequence selected from the group consisting of TCR beta chain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M, is provided. In another embodiment, 1) a TCR alpha chain CDR, TCR V_α domain, and/or TCR alpha chain is encoded by a TRAV, TRAJ, and/or TRAC gene or fragment thereof selected from the group of TRAV, TRAJ, and TRAC genes listed in Table 2, and/or 2) a TCR beta chain CDR, TCR V_β domain, and/or TCR beta chain is encoded by a TRBV, TRBJ, and/or TRBC gene or fragment thereof selected from the group of TRBV, TRBJ, and TRBC genes listed in Table 2, and/or 3) each CDR of the binding protein has up to five amino acid substitutions, insertions, deletions, or a combination thereof as compared to the cognate reference CDR sequence listed in Table 2. In still another embodiment, a binding protein is chimeric, humanized, or human. In yet another embodiment, a binding protein comprises a binding domain having a transmembrane domain, and an effector domain that is intracellular. In another embodiment, a TCR alpha chain and a TCR beta chain are covalently linked, optionally wherein the TCR alpha chain and the TCR beta chain are covalently linked through a linker peptide. In still another embodiment, a TCR alpha chain and/or a TCR beta chain are covalently linked to a moiety, optionally wherein the covalently linked moiety comprises an affinity tag or a label. In yet another embodiment, an affinity tag is selected from the group consisting of aCD34 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, HaloTag, HA tag, Flag tag, His tag, biotin tag, and V5 tag, and/or wherein the label is a fluorescent protein. In another embodiment, a covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic molecule, radioactive isotope, or antibody

or antigen-binding fragment thereof. In still another embodiment, a binding protein binds to the pMHC complex on a cell surface. In yet another embodiment, an MHC or MHC-peptide complex is as described herein. In another embodiment, binding of a binding protein to the MAGEC2 peptide-MHC (pMHC) complex elicits an immune response, optionally wherein the immune response is i) a T cell response and/or a CD8+ T cell response and/or ii) selected from the group consisting of T cell expansion, cytokine release, and/or cytotoxic killing. In still another embodiment, a binding protein is capable of specifically and/or selectively binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex with a K_d less than or equal to about $1x10^{-4}$ M, less than or equal to about $5x10^{-5}$ M, less than or equal to about $1x10^{-5}$ M, less than or equal to about $5x10^{-6}$ M, less than or equal to about $1x10^{-6}$ M, less than or equal to about $5x10^{-7}$ M, less than or equal to about $1x10^{-7}$ M, less than or equal to about $5x10^{-8}$ M, less than or equal to about $1x10^{-8}$ M, less than or equal to about $5x10^{-9}$ M, less than or equal to about $1x10^{-9}$ M, less than or equal to about $5x10^{-10}$ M, less than or equal to about $1x10^{-10}$ M, less than or equal to about $5x10^{-11}$ M, less than or equal to about $1x10^{-11}$ M, less than or equal to about $5x10^{-12}$ M, or less than or equal to about $1x10^{-12}$ M. In yet another embodiment, a binding protein has a higher binding affinity to the peptide-MHC (pMHC) than does a known T-cell receptor, optionally wherein the higher binding affinity is at least 1.05-fold higher. In another embodiment, a binding protein induces higher T cell expansion, cytokine release, and/or cytotoxic killing than does a known T-cell receptor when contacted with target cells with a heterozygous expression of MAGEC2, optionally wherein the induction is at least 1.05-fold higher. As used herein, references to fold changes, in some embodiments, may be in comparison to any reference modality of interest, such as comparison to a different binding protein; comparison tothe same bindng protein under different context like expression of the same binding protein in a different immune cell, at a different level, in combination with other agents described herein; and the like. In still another embodiment, cytotoxic killing is of a target cancer cell. In yet another embodiment, cancer is selected from the group consisting of melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma. In another embodiment, a binding protein does not bind to a peptide-MHC (pMHC) complex comprising a CLHC1 and/or SLC26A7 peptide epitope (*e.g.*, a RVMEYIQQL CLHC1 epitope, a RVMEYIQQ CLHC1 epitope, and/or a RLEALLLSLL SLC26A7 epitope).

These genes are well-known and are recognized to be annotated according to the following NCBI Gene ID numbers, each of which is available on the World Wide Web at ncbi.nlm.nih.gov/gene:

CLHC1: Gene ID 130162 and NM_001135598.2 and NP_001129070.1; NM_001353779.2 and NP_001340708.1; NM_001353780.2 and NP_001340709.1; NM_001353781.2 and NP_001340710.1; NM_001353782.2 and NP_001340711.1; NM_001353783.2 and NP_001340712.1; NM_001353784.2 and NP_001340713.1; NM_001353785.2 and NP_001340714.1; NM_001353786.2 and NP_001340715.1; NM_001353787.2 and NP_001340716.1; and NM_152385.4 and NP_689598.2 as representative clones; and

SLC26A7: Gene ID 115111 and NM_001282356.2 and NP_001269285.1; NM_001282357.2 and NP_001269286.1; NM_052832.4 and NP_439897.1; and NM_134266.2 and NP_599028.1 as representative clones.

In yet another aspect, a TCR alpha chain and/or beta chain selected from the group consisting of TCR alpha chain and beta chain sequences listed in Table 2, is provided.

In another aspect, an isolated nucleic acid molecule i) that hybridizes, under stringent conditions, with the complement of a nucleic acid encoding a polypeptide selected from the group consisting of polypeptide sequences listed in Table 2, ii) a sequence with at least about 80% homology to a nucleic acid encoding a polypeptide selected from the group consisting of the polypeptide sequences listed in Table 2, and/or iii) ii) a sequence with at least about 80% homology to a nucleic acid encoding listed in Table 2, optionally wherein the isolated nucleic acid molecule comprises 1) a TRAV, TRAJ, and/or TRAC gene or fragment thereof selected from the group of TRAV, TRAJ, and TRAC genes listed in Table 2 and/or 2) a TRBV, TRBJ, and/or TRBC gene or fragment thereof selected from the group of TRBV, TRBJ, and TRBC genes listed in Table 2, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a nucleic acid is codon optimized for expression in a host cell.

In still another aspect, a vector comprising an isolated nucleic acid described herein, optionally wherein i) the vector is a cloning vector, expression vector, or viral vector and/or ii) the vector comprises a vector sequence listed in Table 3, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described

herein. For example, in one embodiment, a vector further comprises a nucleic acid sequence encoding CD8α, CD8β, a dominant negative TGFβ receptor II (DN-TGFβRII), and/or a selectable protein marker, optionally wherein the selectable protein marker is dihydrofolate reductase (DHFR). In another embodiment, a nucleic acid sequence encoding CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker is operably linked to a nucleic acid encoding a tag. In still another embodiment, a nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein such that the tag is fused to the N-terminus of CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker. In yet another embodiment, a tag is a CD34 enrichment tag. In another embodiment, an isolated nucleic acid described herein, either alone or in combination with a nucleic acid sequence encoding CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide. In still another embodiment, a self-cleaving peptide is P2A, E2A, F2A or T2A.

In yet another aspect, a host cell which comprises an isolated nucleic acid described herein, comprises a vector described herein, and/or expresses a binding protein described herein, optionally wherein the cell is genetically engineered, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a host cell comprises a chromosomal gene knockout of a TCR gene, an HLA gene, or both. In another embodiment, a host cell comprises a knockout of an HLA gene selected from an α1 macroglobulin gene, α2 macroglobulin gene, α3 macroglobulin gene, β1 microglobulin gene, β2 microglobulin gene, and combinations thereof. In still another embodiment, a host cell comprises a knockout of a TCR gene selected from a TCR α variable region gene, TCR β variable region gene, TCR constant region gene, and combinations thereof. In yet another embodiment, a host cell expresses CD8α, CD8β, a DN-TGFβRII, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR, and further optionally wherein the CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker is fused to a CD34 enrichment tag. In another embodiment, host cells are enriched using the CD34 enrichment tag. In still another embodiment, a host cell is a hematopoietic progenitor cell, peripheral blood mononuclear cell (PBMC), cord blood cell, or immune cell. In yet another embodiment, an immune cell is a T cell, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic

lymphocyte progenitor cell, cytotoxic lymphocyte stem cell, $CD4^+$ T cell, $CD8^+$ T cell, CD4/CD8 double negative T cell, gamma delta ($\gamma\delta$) T cell, natural killer (NK) cell, NK-T cell, dendritic cell, or a combination thereof. In yet another embodiment a T cell is a naive T cell, central memory T cell, effector memory T cell, or a combination thereof. In another embodiment, a T cell is a primary T cell or a cell of a T cell line. In still another embodiment, a T cell does not express or has a lower surface expression of an endogenous TCR. In yet another embodiment, a host cell is capable of producing a cytokine or a cytotoxic molecule when contacted with a target cell that comprises a peptide-MHC (pMHC) complex comprising a MAGEC2 peptide epitope in the context of an MHC molecule. In another embodiment, a host cell is contacted with the target cell *in vitro*, *ex vivo*, or *in vivo*. In still another embodiment, a cytokine is TNF-α, IL-2, and/or IFN-γ. In yet another embodiment, a cytotoxic molecule is perforins and/or granzymes, optionally wherein the cytotoxic molecule is granzyme B. In another embodiment, a host cell is capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with a heterozygous expression of MAGEC2. In still another embodiment, a host cell is capable of producing an at least 1.05-fold higher level of cytokine or a cytotoxic molecule. In yet another embodiment, a host cell is capable of killing a target cell that comprises a peptide-MHC (pMHC) complex comprising the MAGEC2 peptide epitope in the context of an MHC molecule. In another embodiment, killing is determined by a killing assay. In still another embodiment, a ratio of the host cell and the target cell in the killing assay is from 20:1 to 1:4. In yet another embodiment, a target cell is a target cell pulsed with 1 μg/mL to 50 pg/mL of MAGEC2 peptide, optionally wherein the target cell is a cell monoallelic for an MHC matched to the MAGEC2 peptide. In another embodiment, a host cell is capable of killing a higher number of target cells when contacted with target cells with a heterozygous expression of MAGEC2, optionally wherein the cell killing is at least 1.05-fold higher. In still another embodiment, a target cell is cell line or a primary cell, optionally wherein the target cell is selected from the group consisting of a HEK293 derived cell line, a cancer cell line, a primary cancer cell, a transformed cell line, and an immortalized cell line. In yet another embodiment, a MAGEC2 immunogenic peptide is as described herein and/or wherein an MHC or MHC-peptide complex is as described herein. In another embodiment, a host cell does not induce T cell expansion (*e.g.*, proliferation), cytokine release, or cytotoxic killing when contact with a target cell that comprises a peptide-MHC (pMHC) complex comprising a CLHC1 and/or SLC26A7 peptide epitope. In still another embodiment, a host cell does not

express MAGEC2 antigen, is not recognized by a binding protein described herein, is not of serotype HLA-B*07 and/or does not express an HLA-B*07 allele.

In another aspect, a population of host cells described herein, is provided.

In still another aspect, a composition comprising a) a binding protein described herein, b) an isolated nucleic acid described herein, c) a vector described herein, d) a host cell described herein, and/or e) a population of host cells described herein, and a carrier, is provided.

In yet another aspect, a device or kit comprising a) a binding protein described herein, b) an isolated nucleic acid described herein, c) a vector described herein, d) a host cell described herein, and/or e) a population of host cells described herein, said device or kit optionally comprising a reagent to detect binding of a), d) and/or e) to a pMHC complex, is provided.

In another aspect, a method of producing a binding protein described herein, wherein the method comprises the steps of: (i) culturing a transformed host cell which has been transformed by a nucleic acid comprising a sequence encoding a binding protein described herein under conditions suitable to allow expression of said binding protein; and (ii) recovering the expressed binding protein, is provided.

In still another aspect, a method of producing a host cell expressing a binding protein described herein, wherein the method comprises the steps of: (i) introducing a nucleic acid comprising a sequence encoding a binding protein described herein into the host cell; and (ii) culturing the transformed host cell under conditions suitable to allow expression of said binding protein, is provided.

In yet another aspect, a method of detecting the presence or absence of a MAGEC2 antigen and/or a cell expressing MAGEC2, optionally wherein the cell is a hyperproliferative cell, comprising detecting the presence or absence of said MAGEC2 antigen in a sample by use of at least one binding protein described herein, at least one host cell described herein, or a population of host cells described herein, wherein detection of the MAGEC2 antigen is indicative of the presence of a MAGEC2 antigen and/or cell expressing MAGEC2, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, at least one binding protein, or at least one host cell, forms a complex with the MAGEC2 peptide in the context of an MHC molecule, and the complex is detected in the form of fluorescence activated cell sorting (FACS), enzyme linked

immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay. In another embodiment, a method further comprises obtaining a sample from a subject.

In another aspect, a method of detecting the level of a disorder characterized by MAGEC2 expression in a subject, comprising: a) contacting a sample obtained from the subject with at least one binding protein described herein, at least one host cell described herein, or a population of host cells described herein; and b) detecting the level of reactivity, wherein the presence or a higher level of reactivity compared to a control level indicates the level of the disorder characterized by MAGEC2 expression in the subject, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a control level is a reference number. In another embodiment, a control level is a level from a subject without the disorder characterized by MAGEC2 expression.

In still another aspect, a method for monitoring the progression of a disorder characterized by MAGEC2 expression in a subject, the method comprising: a) detecting in a subject sample the presence or level of reactivity between a sample obtained from the subject and at least one binding protein described herein, at least one host cell described herein, or a population of host cells described herein; b) repeating step a) at a subsequent point in time; and c) comparing the level of MAGEC2 or the cell of interest expressing MAGEC2 detected in steps a) and b) to monitor the progression of the disorder characterized by MAGEC2 expression in the subject, wherein an absent or reduced MAGEC2 level or the cell of interest expressing MAGEC2 detected in step b) compared to step a) indicates an inhibited progression of the disorder characterized by MAGEC2 expression in the subject and a presence or increased MAGEC2 level or the cell of interest expressing MAGEC2 detected in step b) compared to step a) indicates a progression of the disorder characterized by MAGEC2 expression in the subject, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a subject has undergone treatment to treat a disorder characterized by MAGEC2 expression between the first point in time and the subsequent point in time.

In yet another aspect, a method for predicting the clinical outcome of a subject afflicted with a disorder characterized by MAGEC2 expression comprising: a) determining

the presence or level of reactivity between a sample obtained from the subject and at least one binding protein described herein, at least one host cell described herein, or a population of host cells described herein; and b) comparing the presence or level of reactivity to that from a control, wherein the control is obtained from a subject having a good clinical outcome; wherein the absence or a reduced level of reactivity in the subject sample as compared to the control indicates that the subject has a good clinical outcome, is provided.

In another aspect, a method of assessing the efficacy of a therapy for a disorder characterized by MAGEC2 expression comprising: a) determining the presence or level of reactivity between a sample obtained from the subject and at least one binding protein described herein, at least one host cell described herein, or a population of host cells described herein, in a first sample obtained from the subject prior to providing at least a portion of the therapy for the disorder characterized by MAGEC2 expression to the subject, and b) determining the presence or level of reactivity between a sample obtained from the subject and at least one binding protein described herein, at least one host cell described herein, or a population of host cells described herein, in a second sample obtained from the subject following provision of the therapy for the disorder characterized by MAGEC2 expression, wherein the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the disorder characterized by MAGEC2 expression in the subject, and wherein the presence or an increased level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious for treating the disorder characterized by MAGEC2 expression in the subject, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a level of reactivity is indicated by a) the presence of binding and/or b) T cell activation and/or effector function, optionally wherein the T cell activation or effector function is T cell proliferation, killing, or cytokine release. In another embodiment, a T cell binding, activation, and/or effector function is detected using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

In still another aspect, a method of preventing and/or treating a disorder characterized by MAGEC2 expression comprising contacting target cells expressing MAGEC2 with a therapeutically effective amount of a composition comprising cells expressing at least one

binding protein described herein, optionally wherein the composition is administered to a subject, is provided.

Numerous embodiments are further provided that may be applied to any aspect encompassed by the present invention and/or combined with any other embodiment described herein. For example, in one embodiment, a cell is an allogeneic cell, syngeneic cell, or autologous cell. In another embodiment, a cell is a host cell described herein or a population of host cells described herein. In still another embodiment, a target cell is a cancer cell expressing MAGEC2. In yet another embodiment, a cell composition further comprises a pharmaceutically acceptable carrier. In another embodiment, a cell composition induces an immune response against the target cell expressing MAGEC2 in the subject. In still another embodiment, a cell composition induces an antigen-specific T cell immune response against the target cell expressing MAGEC2 in the subject. In yet another embodiment, an antigen-specific T cell immune response comprises at least one of a $CD4^+$ helper T lymphocyte (Th) response and a CD8+ cytotoxic T lymphocyte (CTL) response. In another embodiment, a method further comprises administering at least one additional treatment for the disorder characterized by MAGEC2 expression, optionally wherein the at least one additional treatment for the disorder characterized by MAGEC2 expression is administered concurrently or sequentially with the composition. In still another embodiment, a disorder characterized by MAGEC2 expression is a cancer or relapse thereof, optionally wherein the cancer is selected from the group consisting of melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma. In yet another embodiment, a subject is an animal model of a disorder characterized by MAGEC2 expression and/or a mammal, optionally wherein the mammal is a human, a primate, or a rodent.

Brief Description of the Drawings

Certain working examples and figures refer to certain control TCRs, such as "Leiden-8-3" which corresponds to a TCR described in U.S. Ser. No. 18/286,728, PCT Pat. Appl. No. PCT/US2022/024728, and PCT Publ. No. WO 2022/221479 (see, *e.g.*, Table 2A of each document), each of which is incorporated herein in its entirety by this reference, and described further herein such as at Table 4.

Figure 1 shows results of a representative example cytotoxicity assay used to select codon-optimized TCRs for cytotoxicity against MAGEC2 expressing cell lines. Donor T cells were co-cultured with the B*07:02 and MAGEC2 expressing cell line FTC133 at an

effector to target (E:T) ratio of 1:5. FTC133 cells were labeled with Incucyte® NucLight™ Red and cytotoxicity activity of the TCR was measured by loss of fluorescence of the target cell line by with an Incucyte® system.

Figure 2 shows results of a representative example cytotoxicity assay used to compare the efficacy of the top codon optimized TCRs identified from the screen described in Figure 1. TCR T cells were generated by transducing with the indicated TCR and selecting for TCR expression to normalize reactivity. Isolated cells were co-cultured at an E:T of 5:1 with the indicated cell lines that express either high (FTC133), moderate (A101D), low (SKLMS1) levels of MAGEC2 or are negative for MAGEC2 expression (A2058).

Figures 3A-3D show cytokine release in response to co-culture with cell lines expressing various levels of MAGEC2 as in Figure 2. Release of granzyme (Figure 3A), IFNgamma (Figure 3B), TNFalpha (Figure 3C) and IL-2 (Figure 3D) was measured from the supernatant after 24 hours of co-culture using an ELLA® system.

Figures 4A and **4B** show the pMHC dose-dependent function of process-representative TSC-201-B0702 TCR-T cells. T2-B07 cells were pulsed with various concentrations of the MAGE-C2 peptide and co-cultured with two batches of TSC-201-B0702 process-representative TCR-T cells. Figures 4A and 4B show the relative growth of T2-B07 cells over 72 hours of co-culture with TSC-201-B0702 TCR-T cells at an E:T ratio of 5:1, normalized to t=0h. For each donor, the co-culture was performed in triplicate (n=3). The error bars at each data point show the standard error of the mean (SEM). The area under the curve (AUC) for the resulting growth of the T2-B07 cells over 72 has a function of the peptide concentration was plotted to compare the batches of TSC-201-B0702.

Figures 5A-5H show that TSC-201-B0702 TCR-T cells secreted Granzyme B and inflammatory cytokines IFN-γ, IL-2 and TNF-α in a target-dependent manner. TSC-201-B0702 TCR-T cells (Figures 5A-5D) or donor-matched untransfected (UTF) control T cells (Figures 5E-5H) from four donors (PD353, PD354, PD355 and PD356) were cultured in the absence of targets cells (black bars), or were co-cultured at an E:T of 1:1 with either the HLA-B*07:02 positive, MAGE-C2-negative target cell line A2058 (grey bars) or three different HLA-B*07:02-positive MAGE-C2-positive cell lines (A101D, medium green bars; FTC-133, light green bars; COLO-783, dark green bars). Supernatants were collected after 24-hour co-culture and levels of inflammatory cytokines IFN-γ, IL-2 and TNF-α as well as Granzyme B were assessed with an automated 4-plex ELISA assay (ELLA from Protein Simple). Co-cultures were prepared in triplicate; the error bars at each data point show the standard error of the mean (SEM). # Denotes samples that were outside of the dynamic range

of the assay (>20000 pg/mL for IFN-γ, >25000 pg/mL for Granzyme B), and & denotes samples for which values were beneath the detection limit.

Figures 6A and **6B** show that TSC-201-B0702 TCR-T cells proliferated in a target-dependent manner. TSC-201-B0702 TCR-T cells (Figure 12A) or donor-matched untransfected control T cells (Figure 6B) from four T cell batches (PD353, PD354, PD355 and PD356) were labeled with CTV dye and were cultured in the absence of targets cells (black bars), or were co-cultured at an E:T of 1:1 with either the HLA-B*07:02-positive, MAGE-C2-negative target cell line A2058 (light bars) or three different HLA-B*07:02-positive MAGE-C2-positive cell lines (A101D, medium green; FTC-133, light green bars; COLO-783, dark green bars). After 3.5-day co-culture, cells were stained for flow cytometric quantification of T cell proliferation. Graphs depict the number of dividing cells (identified as CTV dim population) normalized to counting beads. Co-cultures were prepared in triplicate; the error bars at each data point show the standard error of the mean (SEM). The number of dividing cells is shown for the following T cell subsets: total T cells (left panels); helper T cells (middle panels) and cytotoxic T cells (right panels).

Figures 7A and **7B** show that TSC-201-B0702 TCR-T cells displayed potent and selective cytotoxicity. Figure 13A shows that four batches of process-representative TSC-201-B0702 TCR-T cells (green growth curves) and untransfected (UTF) control T cells from matched donors (gray growth curves) were analyzed in the Incucyte®-based cytotoxicity assay for their cytotoxicity potential against an HLA-B*07:02-positive, MAGE- C2-negative control cell line (A2058) or three different HLA-B*07:02-positive, MAGE-C2-positive target cell lines (COLO-783, FTC-133, and A101D). T cells and target cells were co-cultured across a range of effector to target ratios (E:T ranging from 5:1 to 0.6:1) and the growth of the target cells was measured over 72 hours. Data presented were obtained with TSC-201-B0702 TCR-T cells and UTF control T cells from batch PD354 and are representative of the data obtained with all 4 batches of process-representative material tested. Target cells cultured alone are displayed as a negative control (red growth curves). Figure 13B shows that the cytotoxic activity of the four batches of process-representative TSC-201-B0702 TCR-T cells over 72 hours is summarized as the area under the curve (AUC) of the growth curves of target cells co-cultured with TSC-201-B0702 TCR-T cells at an E:T of 2.5:1, normalized to the growth curves of target cells co-cultured with the corresponding UTF control cells. Co-cultures were performed in triplicate; bars depict mean with standard error.

Figure 8 shows the histogram analysis of phospho-SMAD2 or total SMAD2 in TSC-201-B0702 TCR- T or UTF cells. Shown are data obtained with TSC-201-B0702 process-

representative TCR-T cells (batches PD353, PD354 and PD355) and bench-scale process-similar UTF cells from the same donors (PD353, PD354) and a third unrelated batch (D6446) untreated or treated with 10 ng/mL TGFβ-1. Viable single cells are characterized as CD34-positive (TSC-201-B0702 TCR-T cells) or CD34-negative (UTF controls) and these subpopulations are further characterized by histogram analysis for phospho-SMAD2 or total SMAD2. H istogram analysis overlays consist of phospho-SMAD2 or total SMAD2 in untreated CD34 positive-TCR-T cells (dark green, filled), TGFβ treated CD34 positive-TCR-T cells (dark green, open), untreated CD34 negative-UTF (black, filled) and TGFβ treated CD34 negative-UTF (black, open).

Figure 9 shows the inoculation, dosing, and analyses schedule.

Figures 10A-10E show the TSC-201-B0702 *in vivo* efficacy. NCG mice were inoculated *s.c.* with A101D β2M tumor cells. Once tumor engraftment was successful (5 days post inoculation), animals were randomized into 7 different treatment groups and injected with process-representative TSC-201-B0702 TCR-T cells (3 batches tested, PD353, PD354, and PD355); or untransfected (UTF) control T cells from matched donors, or vehicle (PBS) on day 1, and 8 of the study (triangle heads). Mean tumor volume of each treatment group of mice (n=12) over time is shown (Figure 10A). Area under the curve (AUC) value representing tumor growth curves for individual mice for each treatment group between day 0 and 28 is shown (Figure 10B). Tumor volume of individual mice over time is shown (Figures. 16C-16E). ****P<0.0001, compared to respective UTF control groups. Error bars represent SEM.

Figure 11 shows the percentage of body weight evolution over time across the different groups. NCG mice were inoculated *s.c.* with A101D B2M tumor cells. Once tumor engraftment was successful (5 days post inoculation), animals were randomized into 7 different treatment groups and injected with process-representative TSC-201-B0702 TCR-T cells (3 batches tested, PD353, PD354, and PD355); or of untransfected (UTF) control T cells from matched donors, or of vehicle (PBS) on day 1, and 8 of the study (triangle heads). The % body weight change for all the treatment groups(n=12) is shown. Error bars represent SEM of mean.

Figure 12 is schematic illustrating the principle of the T-Scan screen.

Figure 13 is graphical representation of the results of the T-Scan screen for mechanistically representative TSC-201-B0702 TCR-T cells. Plotted is the enrichment score for each of ~500,000 tiles/peptides in the screen calculated from 8 technical replicates, measured relative to the input. Tiles that are enriched above background are highlighted in

colors, with overlapping tiles displayed in matching colors, and the corresponding protein names are indicated on the graph.

Figure 14 is alloreactivity profiling of mechanistically representative TSC-201-B0702 TCR-T cells. Mechanistically representative TSC-201-B0702 TCR-T cells were co-cultured with MHC-null HEK293T cells re-expressing one of the 110 most frequently encountered Class I HLAs in the US population for 48h. A positive control consisting of HEK293T cells expressing both a fragment of MAGE-C2 containing the HLA-B*07:02-restricted epitope and HLA-B*07:02 (red) and a negative control consisting of MHC-null HEK293T cells (blue) were included in the screen. The inhibition of target cell growth by the TCR-T cells relative to that by the UTD control T cells was measured over 48 hours of co-culture as a readout of the reactivity of mechanistically representative version of the therapeutic TCR to allogeneic HLA proteins.

Figure 15 shows the flow chart describing the steps and timelines of the cytokine assay to test off-tumor reactivity of TSC-201-B0702 TCR-T cell.

Figure 16 shows the expression of the putative off-targets of the therapeutic TCR used in TSC-201- B0702 TCR-T cells in primary and iPSC-derived cells. RNA was extracted from the primary and iPSC-derived cells and sequenced. Heat maps show TPM (transcripts per million) calculated from the counts. The color scale used in RNAseq heatmaps has TPM values of zero set to white and values above zero follow a continuous color scale up to 100 TPM.

Figures 17A-17C show that TSC-201-B0702 TCR-T cells show no reactivity to HLA-B*07:02$^+$ primary cells. TSC-201-B0702 TCR-T cells and donor-matched UTF cells were co-cultured with a panel of primary cells and supernatants were evaluated for levels of IFN-γ as a measure of T cell reactivity. # denotes samples that were outside of the dynamic range of the assay (>20,000 pg/mL for IFN-γ).

Figure 18 shows the expression of the putative off-targets of the therapeutic TCR used in TSC-201- B0702 TCR-T cells in cancer cell lines. RNA was extracted from the cancer cell lines and sequenced. Heat maps show TPM (transcripts per million) calculated from the counts. The color scale used in RNAseq heatmaps has TPM values of zero set to white and values above zero follow a continuous color scale up to 100 TPM.

Figures 19A-19C show that TSC-201-B0702 TCR-T cells showed no reactivity to HLA-B*07:02$^+$ cancer cell lines. TSC-201-B0702 TCR-T cells and donor-matched UTF cells were co-cultured with a panel of cancer cell lines and supernatants were evaluated for levels of IFN-γ as a measure of T cell reactivity. # denotes samples that were outside of the

dynamic range of the assay (>20,000 pg/mL for IFN-γ).

Figure 20 shows MAGEC2 expression in 48 normal human organs.

Figures 21A-21E show annotated vector maps for vector sequences provide in Table 3 (Figures 21A-21D) and Table 4 (Figure 21).

For any figure showing a bar histogram, curve, or other data associated with a legend, the bars, curve, or other data presented from left to right for each indication correspond directly and in order to the boxes from top to bottom, or from left to right, of the legend unless indicated otherwise.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of MAGEC2 immunogenic peptides (*e.g.*, those comprising or consisting of sequences listed in Table 1), binding proteins (*e.g.*, those having sequences listed in Table 2) that recognize MAGEC2 antigens, and uses thereof. A systematic, comprehensive survey was carried out to map the precise T cell targets recognized by an initial pool of T cells of interest.

Accordingly, the present invention relates, in part, to the identified epitopes (immunodomiannt peptides) of therapeutically relevant MAGEC2 protein and related compositions (*e.g.*, immunodominant peptides, vaccines, and the like), compositions comprising immunogenic peptides alone or with MHC molecules, stable MHC-peptide complexes, methods of diagnosing, prognosing, and monitoring immune responses to disorders characterized by MAGEC2 expression, and methods for preventing and/or treating disorders characterized by MAGEC2 expression. The present invention also relates, in part, to identified binding proteins (*e.g.*, TCRs), host cells expressing binding proteins (*e.g.*, TCRs), compositions comprising binding proteins (*e.g.*, TCRs) and host cells expressing binding proteins (*e.g.*, TCRs), methods of diagnosing, prognosing, and monitoring T cell response to cells expressing MAGEC2, and methods for preventing and/or treating disorders characterized by MAGEC2 expression.

I. Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means

one element or more than one element. In addition, references to a table provided herein encompass all sub-tables of the table unless otherwise indicated.

The term "administering" means providing a pharmaceutical agent or composition to a subject, and includes, but is not limited to, administering by a medical professional and self-administering. This involves the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. In some embodiments, routes of administration for binding proteins described herein include intravenous, intraperitoneal, intramuscular, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intraperitoneal, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. Alternatively, a binding protein described herein may be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering may also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

As used herein, the term "antigen" refers to any natural or synthetic immunogenic substance, such as a protein, peptide, or hapten. An antigen may be a MAGEC2 antigen, or a fragment thereof, against which protective or therapeutic immune responses are desired. An "epitope" is the part of the antigen bound by a natural or synthetic substance.

The term "adjuvant" as used herein refers to substances, which when administered prior, together or after administration of an antigen accelerates, prolong and/or enhances the quality and/or strength of an immune response to the antigen in comparison to the administration of the antigen alone. Adjuvants can increase the magnitude and duration of the immune response induced by vaccination.

The term "antibody" as used to herein includes whole antibodies and any antigen binding fragments (*i.e.*, "antigen-binding portions") or single chains thereof. An "antibody" refers, in one embodiment, to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as V_H) and a heavy chain constant region. In certain naturally occurring antibodies, the heavy chain

constant region is comprised of three domains, CH1, CH2 and CH3. In certain naturally occurring antibodies, each light chain is comprised of a light chain variable region (abbreviated herein as V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

The term "antigen presenting cell" or "APC" includes professional antigen presenting cells (*e.g.*, B lymphocytes, monocytes, dendritic cells, Langerhans cells), as well as other antigen presenting cells (*e.g.*, keratinocytes, endothelial cells, astrocytes, fibroblasts, and oligodendrocytes).

The term "antigen-binding portion" of a binding protein, such as a TCR, as used herein, refers to one or more portions of a TCR that retain the ability to bind (*e.g.*, specifically and/or selectively) to an antigen (*e.g.*, a MAGEC2 antigen). Such portions are, for example, between about 8 and about 1,500 amino acids in length, suitably between about 8 and about 745 amino acids in length, suitably about 8 to about 300, for example about 8 to about 200 amino acids, or about 10 to about 50 or 100 amino acids in length. It has been shown that the antigen-binding function of a TCR can be performed by fragments of a full-length TCR. Examples of binding portions encompassed within the term "antigen-binding portion" of a TCR, include (i) a Fv fragment consisting of the V_α and V_β domains of a TCR, (ii) an isolated complementarity determining region (CDR) or (iii) a combination of two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although V_α and V_β, are coded by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_α and V_β regions pair to form monovalent molecules (known as single chain TCR (scTCR)). Such single chain TCRs are also intended to be encompassed within the term "antigen-binding portion" of a TCR. These TCR fragments can be obtained using conventional techniques known to those with skill in the art, and the fragments are screened

for utility in the same manner as are complete binding proteins. Antigen-binding portions may be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins.

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 certain binding proteins, such as TCR variable regions, which confer antigen specificity and/or binding affinity. For TCRs, in general, there are three CDRs in each α-chain variable region (αCDR1, αCDR2, and αCDR3) and three CDRs in each β-chain variable region (βCDR1, βCDR2, and βCDR3). CDR3 is believed to be the main CDR responsible for recognizing processed antigen. CDR1 and CDR2 mainly interact with the MHC.

The term "body fluid" refers to fluids that are excreted or secreted from the body as well as fluids that are normally not excreted or secreted from the body (*e.g.*, amniotic fluid, aqueous humor, bile, blood and blood plasma, cerebrospinal fluid, cerumen and earwax, cowper's fluid or pre-ejaculatory fluid, chyle, chyme, stool, female ejaculate, interstitial fluid, intracellular fluid, lymph, menses, breast milk, mucus, pleural fluid, pus, saliva, sebum, semen, serum, sweat, synovial fluid, tears, urine, vaginal lubrication, vitreous humor, vomit). In some embodiments, the body fluid comprises immune cells, optionally wherein the immune cells are cytotoxic lymphocytes such as cytotoxic T cells and/or NK cells, CD4+ T cells, and the like.

The term "coding region" refers to regions of a nucleotide sequence comprising codons that are translated into amino acid residues, whereas the term "non-coding region" refers to regions of a nucleotide sequence that are not translated into amino acids (*e.g.*, 5' and 3' untranslated regions).

The term "comparator T-cell receptor" refers to at least one benchmark T-cell receptor. In some embodiments, a comparator TCR is the Leiden-8-3 TCR, which corresponds to a TCR described in U.S. Ser. No. 18/286,728, PCT Pat. Appl. No. PCT/US2022/024728, and PCT Publ. No. WO 2022/221479 (see, *e.g.*, Table 2A of each document), each of which is incorporated herein in its entirety by this reference. Engineered versions of such parental sequences were used in the working examples and sequences of such engineered versions are set forth in Table 4. In some embodiments, the comparator T-cell receptor has sequences set forth in Table 4.

The term "complementary" refers to the broad concept of sequence complementarity between regions of two nucleic acid strands or between two regions of the same nucleic acid strand. It is known that an adenine residue of a first nucleic acid region is capable of forming specific hydrogen bonds ("base pairing") with a residue of a second nucleic acid region which is anti-parallel to the first region if the residue is thymine or uracil. Similarly, it is known that a cytosine residue of a first nucleic acid strand is capable of base pairing with a residue of a second nucleic acid strand which is anti-parallel to the first strand if the residue is guanine. A first region of a nucleic acid is complementary to a second region of the same or a different nucleic acid if, when the two regions are arranged in an antiparallel fashion, at least one nucleotide residue of the first region is capable of base pairing with a residue of the second region. In some embodiments, the first region comprises a first portion and the second region comprises a second portion, whereby, when the first and second portions are arranged in an antiparallel fashion, at least about 50%, and, in other embodiments, at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or any range in between, inclusive, such as at least about 80%-100%, of the nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion. In some embodiments, all nucleotide residues of the first portion are capable of base pairing with nucleotide residues in the second portion.

As used herein, the term "costimulate" with reference to activated immune cells includes the ability of a costimulatory molecule to provide a second, non-activating receptor mediated signal (a "costimulatory signal") that induces proliferation or effector function. For example, a costimulatory signal may result in cytokine secretion, *e.g.,* in a T cell that has received a T cell-receptor-mediated signal. Immune cells that have received a cell-receptor mediated signal, *e.g.,* via an activating receptor are referred to herein as "activated immune cells."

"CD3" is known in the art as a multi-protein complex of six chains (see, Abbas and Lichtman, Cellular and Molecular Immunology (9^{th} Edition) (2018); Janeway *et al.* (Immunobiology) (9^{th} Edition) (2016)). 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 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 is believed to allow these chains

to associate with positively charged regions or residues of 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 IT AM, whereas each CD3ζ chain has three ITAMs. Without wishing to be bound by theory, it is believed that the IT AMs are important for the signaling capacity of a TCR complex. CD3 used in accordance with the present invention may be from various animal species, including human, mouse, rat, or other mammals.

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).

The term "chimeric antigen receptor" or "CAR" refers to a fusion protein that is engineered to contain two or more amino acid sequences linked together in a way that does not occur naturally or does not occur naturally in a host cell, which fusion protein can function as a receptor when present on a surface of a cell. CARs encompassed by the present invention include an extracellular portion comprising an antigen-binding domain (*i.e.,* obtained or derived from an immunoglobulin or immunoglobulin-like molecule, such as a TCR specific for a MAGEC2 antigen, a single chain TCR-derived binding protein, an scFv derived from an antibody, an antigen binding domain derived or obtained from a killer immunoreceptor from an NK cell, and the like) linked to a transmembrane domain and one or more intracellular signaling domains (such as an effector domain, optionally containing co-stimulatory domain(s)) (see, *e.g.,* Sadelain *et al.* (2013) *Cancer Discov.* 3:388; see also Harris and Kranz (2016) *Trends Pharmacol. Sci.* 37: 220; Stone *et al.* (2014) *Cancer Immunol. Immunother.* 63:1163).

As used herein, the term "cytotoxic T lymphocyte (CTL) response" refers to an immune response induced by cytotoxic T cells. CTL responses are mediated primarily by $CD8^+$ T cells.

The term "consisting essentially of is not equivalent to "comprising" and refers to the specified materials or steps of a claim, or to those that do not materially affect the basic characteristics of a claimed subject matter. For example, a protein domain, region, or module (*e.g.,* a binding domain, hinge region, linker module) or a protein (which may have one or more domains, regions, or modules) "consists essentially of a particular amino acid sequence

when the amino acid sequence of a domain, region, module, or protein includes extensions, deletions, mutations, or a combination thereof (*e.g.*, amino acids at the amino- or carboxy - terminus or between domains) that, in combination, contribute to at most 20% (*e.g.*, at most 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2% or 1%) of the length of a domain, region, module, or protein and do not substantially affect (*i.e.*, do not reduce the activity by more than 50%, such as no more than 40%, 30%, 25%, 20%, 15%, 10%, 5%, or 1%) the activity of the domain(s), region(s), module(s), or protein (*e.g.*, the target binding affinity of a binding protein).

The term "determining a suitable treatment regimen for the subject" is taken to mean the determination of a treatment regimen (*i.e.*, a single therapy or a combination of different therapies that are used for the prevention and/or treatment of the viral infection in the subject) for a subject that is started, modified and/or ended based or essentially based or at least partially based on the results of the analysis according to the present invention. One example is starting an adjuvant therapy after surgery whose purpose is to decrease the risk of recurrence, another would be to modify the dosage of a particular chemotherapy. The determination can, in addition to the results of the analysis according to the present invention, be based on personal characteristics of the subject to be treated. In most cases, the actual determination of the suitable treatment regimen for the subject will be performed by the attending physician or doctor.

The term "dominant negative TGFβ receptor" or "DN-TGFβR" refers to a transforming growth factor (TGF) beta receptor variant or mutant that provides resistance to TGFβ signaling. There are five type II receptors (activation receptors) and seven type I receptors (signaling propagation receptors). The active TGFβ receptor is a heterotetramer consisting of two TGF β receptors I (TGFβRI) and two TGF β receptors II (TGFβRII). In some embodiments, the DN-TGFβR is a DN-TGFβRII (*i.e.*, a TGF beta receptor II variant or mutant). In some embodiments, resistance is to the suppressive effect of TGFβ signaling on an immune cell, such as a T cell, which TGFβ may be produced by cancer cells or by other immune cells within a cellular environment, such as by stromal cells, macrophages, myeloid cells, epithelial cells, natural killer cells, and the like. TGFβ signaling inhibitors are well-known in the art and include, without limitation, mutant TGFβ that sequesters receptors and thereby inhibits signaling, antibodies that bind to TGFβ and/or TGFβ receptors (*e.g.*, lerdelimumab, metlimumab, fressolimumab, and the like), soluble TGFβ-binding proteins such as portions of TGFβ receptors that sequester TGFβ (*e.g.*, TGFβRII-Fc fusion proteins)

or other binders, such as beta-glycans. Any and all known TGFβ signaling inhibitors may be used instead of or in addition to DN-TGFβR (*e.g.*, DN-TGFβRII) described herein. In some embodiments, a DN-TGFβR lacks an intracellular portion required for TGFβ-mediated signaling, such as the entire intracellular domain, a kinase signaling domain, etc. DN-TGFβR constructs are well-known in the art (see representative, non-limiting embodiments at Brand *et al.* (1993) *J. Biol. Chem.* 268:11500-11503; Weiser *et al.* (1993) *Mol. Cell Biol.* 13:7239-7247; Bollard *et al.* (2002) *Blood* 99::3179-3187; PCT Publ. WO 2009/152610; PCT Publ. WO 2017/156484; Kloss *et al.* (2018) *Mol. Ther.* 26:1855-1866; PCT Publ. WO. 2019/089884; PCT Publ. WO 2020/042647; and PCT Publ. WO 2020/042648.

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).

"Homologous" as used herein, refers to nucleotide sequence similarity between two regions of the same nucleic acid strand or between regions of two different nucleic acid strands. When a nucleotide residue position in both regions is occupied by the same nucleotide residue, then the regions are homologous at that position. A first region is homologous to a second region if at least one nucleotide residue position of each region is occupied by the same residue. Homology between two regions is expressed in terms of the proportion of nucleotide residue positions of the two regions that are occupied by the same nucleotide residue. By way of example, a region having the nucleotide sequence 5'-ATTGCC-3' and a region having the nucleotide sequence 5'-TATGGC-3' share 50% homology. In some embodiments, the first region comprises a first portion and the second region comprises a second portion, whereby, at least about 50%, and, in other embodiments, at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or any range in between, inclusive, such as at least about 80%-100%, of the nucleotide residue positions of each of the portions are occupied by the same nucleotide residue. In some embodiments, all nucleotide residue positions of each of the portions are occupied by the same nucleotide residue.

The term "hyperproliferative disorder characterized by expression of a MAGEC2 antigen" can be any hyperproliferative disorder where the MAGEC2 antigen is present in a

MHC (*e.g.*, HLA) complex expressed by at least some hyperproliferating cells in the subject. Examples of hyperproliferative disorders characterized by MAGEC2:HLA complexes include solid malignancies, such as those described in detail *infra*.

The term "immune response" includes T cell mediated and/or B cell mediated immune responses. Exemplary immune responses include T cell responses, *e.g.*, cytokine production and cellular cytotoxicity. In addition, the term immune response includes immune responses that are indirectly effected by T cell activation, *e.g.*, antibody production (humoral responses) and activation of cytokine responsive cells, *e.g.*, macrophages.

An increased ability to stimulate an immune response or the immune system, can result from an enhanced agonist activity of T cell costimulatory receptors and/or an enhanced antagonist activity of inhibitory receptors. An increased ability to stimulate an immune response or the immune system may be reflected by a fold increase of the EC_{50} or maximal level of activity in an assay that measures an immune response, *e.g.*, an assay that measures changes in cytokine or chemokine release, cytolytic activity (determined directly on target cells or indirectly via detecting CD107a or granzymes) and proliferation. The ability to stimulate an immune response or the immune system activity may be enhanced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, 250%, 300%, 350%, 400%, 500%, or more.

The term "immunotherapeutic agent" may include any molecule, peptide, antibody or other agent which can stimulate an immune system to generate an immune response to a cancer cell, such as in a subject. Various immunotherapeutic agents are useful in the compositions and methods described herein.

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

An "isolated protein" refers to a protein that is substantially free of other proteins, cellular material, separation medium, and culture medium when isolated from cells or produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the binding protein, antibody, polypeptide, peptide or fusion protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a biomarker polypeptide or fragment thereof, in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a biomarker protein or fragment thereof, having less than about 30% (by dry weight) of non-biomarker protein (also referred to herein as a "contaminating protein"), or, in some embodiments, less than about 25%, 20%, 15%, 10%, 5%, 1%, or less, or any range in between inclusive, such as less than about 1% to 5%, of non-biomarker protein. When binding protein, antibody, polypeptide, peptide or fusion protein or fragment thereof, *e.g.*, a biologically active fragment thereof, is recombinantly produced, it may be substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 15%, 10%, 5%, 1%, or less, or any range in between inclusive, such as less than about 1% to 5%, of the volume of the protein preparation.

As used herein, the term "isotype" refers to the antibody class (*e.g.*, IgM, IgG1, IgG2C, and the like) that is encoded by heavy chain constant region genes.

As used herein, the term "K_D" is intended to refer to the dissociation equilibrium constant of a particular binding protein-antigen interaction. The binding affinity of binding proteins encompassed by the present invention may be measured or determined by standard binding protein-target binding assays, for example, competitive assays, saturation assays, or standard immunoassays, such as ELISA or RIA. A relatively lower Kd value indicates a relatively higher binding affinity (*e.g.*, Kd values of less than or equal to about $5x10^{-4}$ M (500 uM) include a Kd value of $1x10^{-4}$ M (100 uM) and a 100 uM Kd indicates a relatively higher binding affinity as compared to a 500 uM Kd).

A "kit" is any manufacture (*e.g.*, a package or container) comprising at least one reagent, *e.g.*, a probe or small molecule, for specifically detecting and/or affecting the expression of a marker encompassed by the present invention. The kit may be promoted, distributed, or sold as a unit for performing the methods encompassed by the present

invention. The kit may comprise one or more reagents necessary to express a composition useful in the methods encompassed by the present invention. In some embodiments, the kit may further comprise a reference standard, *e.g.*, a nucleic acid encoding a protein that does not affect or regulate signaling pathways controlling cell growth, division, migration, survival or apoptosis. One skilled in the art can envision many such control proteins, including, but not limited to, common molecular tags (*e.g.*, green fluorescent protein and beta-galactosidase), proteins not classified in any of pathway encompassing cell growth, division, migration, survival or apoptosis by GeneOntology reference, or ubiquitous housekeeping proteins. Reagents in the kit may be provided in individual containers or as mixtures of two or more reagents in a single container. In addition, instructional materials which describe the use of the compositions within the kit may be included.

As used herein, the term "linked" refers to the association of two or more molecules. The linkage may be covalent or non-covalent. The linkage also may be genetic (*i.e.*, recombinantly fused). Such linkages may be achieved using a wide variety of art recognized techniques, such as chemical conjugation and recombinant protein production.

A "linker," in some embodiments, may refer 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 some 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.

The term "MAGEC2" refers to a particular member of the melanoma antigen gene family clustered on human chromosome Xq26-q27 that is also known as cancer/testis antigen 10 (CT10), hepatocellular cancer antigen 587 (HCA587), and melanoma antigen, family E, 1, cancer/testis specific (MAGEE1) (Gure *et al.* (2000) *Int. J. Cancer* 85:726-732; Li *et al.* (2003) *Lab Invest.* 83:1185-1192; Ma *et al.* (2004) *Int. J. Cancer* 109:698-702; Godelaine *et al.* (2007) *Cancer Immunol. Immunother.* 56:753-759; Reiner *et al.* (2009) *Int. J. Cancer* 124:352-357; Doyle *et al.* (2010) *Mol. Cell* 39:93-974; von Boehmer *et al.* (2011) *PLoS One* 6, e21366; Wen *et al.* (2011) *Cancer Sci.* 102:1455-1461; de Carvalho *et al.* (2013) *Cancer Immunol. Immunother.* 62:191-195; Bhatia *et al.* (2013) *J. Invest. Dermatol.* 133:759-767); Yang *et al.* (2014) *Breast Cancer Res. Treat.* 145:23-32; and Kunert *et al.* (2016) *J. Immunol.* 197:2541-2552). MAGEC2 is believed to enhance ubiquitin ligase activity of RING-type zinc finger-containing E3 ubiquitin-protein ligases, such as by recruiting and/or stabilizing

Ubl-conjugating enzymes (E2) at the E3:substrate complex. MAGEC2 enhances *in vitro* ubiquitin ligase activity of TRIM28 and stimulates p53/TP53 ubiquitination in presence of Ubl-conjugating enzyme UBE2H leading to p53/TP53 degradation. MAGEC2 is not expressed in normal tissues, except for testis, and is expressed in tumors of various histological types such as melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma.

The term "MAGEC2" is intended to include fragments, variants (*e.g.*, allelic variants), and derivatives thereof. Representative human MAGEC2 cDNA and human MAGEC2 protein sequences are well-known in the art and are publicly available from the National Center for Biotechnology Information (NCBI) (see, for example, ncbi.nlm.nih.gov/gene/51438). For example, human MAGEC2 (NP_057333.1) is encodable by the transcript (NM_016249.4). Nucleic acid and polypeptide sequences of MAGEC2 orthologs in organisms other than humans are well-known and include, for example, chimpanzee MAGEC2 (NM_001302428.1, NP_001289357.1, XM_016942653.1, and XP_016798142.1) and rhesus monkey MAGEC2 (NM_001265825.1, NP_001252754.1, XM_028841693.1, and XP_028697526.1). Representative sequences of MAGEC2 sequences are also presented below in Table 3.

Anti-MAGEC2 antibodies suitable for detecting MAGEC2 protein are well-known in the art and include, for example, antibodies TA315476 and TA342769 (OriGene, Rockville, MD); antibodies orb353181 and orb 125944 (Biorbyt, Cambridge, United Kingdom); antibodies A05335 and AO5335-1 (Boster Bio, Pleasanton, CA); and antibodies ABIN2788251 and ABIN2706502 (Antibodies-online, Limerick, PA). In addition, reagents are well-known for detecting MAGEC2 expression. Moreover, multiple siRNA, shRNA, CRISPR constructs for modulating MAGEC2 expression can be found in the commercial product lists of a variety of companies, such as open reading frame (ORF) clones SC07208 and RN211555 (OriGene, Rockville, MD) and CRISPR knockouts GA109805 and KN403064 (OriGene, Rockville, MD). It is to be noted that the term can further be used to refer to any combination of features described herein regarding MAGEC2 molecules. For example, any combination of sequence composition, percentage identify, sequence length, domain structure, functional activity, *etc.* can be used to describe a MAGEC2 molecule encompassed by the present invention.

The term "MAGEC2 antigen" or "MAGEC2 peptide antigen" or "MAGEC2-containing peptide antigen" or "MAGEC2 epitope" or "MAGEC2 peptide epitope" or

"MAGEC2 peptide" refers to a naturally or synthetically produced immunogenic portion of MAGEC2. In some embodiments, MAGEC2 antigen protein can range in length from about 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 amino acids, or any range in between, inclusive, such as 8-15 amino acids. In some embodiments, MAGEC2 antigen protein can form a complex with an MHC (*e.g.*, HLA) molecule such that a binding protein of this disclosure that recognizes a MAGEC2 peptide:MHC (*e.g.*, HLA) complex can bind (*e.g.*, specifically and/or selectively) to such a complex. Representative MAGEC2 peptide antigen sequences are shown in Table 1.

The term "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 a chain (with three a domains) and a non-covalently associated b2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, a and b, 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 antigen-MHC (pMHC) complex is recognized by $CD8^+$ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by $CD4^+$ T cells. Human MHC is referred to as human leukocyte antigen (HLA).

The terms "prevent," "preventing," "prevention," "prophylactic treatment," and the like refer to reducing the probability of developing a disease, disorder, or condition in a subject, who does not have, but is at risk of or susceptible to developing a disease, disorder, or condition.

The term "prognosis" includes a prediction of the probable course and outcome of a cancer or the likelihood of recovery from the disease. In some embodiments, the use of statistical algorithms provides a prognosis of a cancer in an individual. For example, the prognosis may be surgery, development of a clinical subtype of a cancer, development of one or more clinical factors, or recovery from the disease.

As used herein, "percent identity" between amino acid sequences is synonymous with "percent homology," which can be determined using the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified by Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. The noted algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410. BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=12, to obtain nucleotide sequences homologous to a polynucleotide described herein. BLAST protein searches are performed with the XBLAST program, score=50,

wordlength=3, to obtain amino acid sequences homologous to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul *et al.* (1997) *Nuc. Acids Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.,* XBLAST and NBLAST) may be used.

The phrase "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body.

The term "ratio" refers to a relationship between two numbers (*e.g.*, scores, summations, and the like). Although, ratios may be expressed in a particular order (*e.g.*, a to b or a:b), one of ordinary skill in the art will recognize that the underlying relationship between the numbers may be expressed in any order without losing the significance of the underlying relationship, although observation and correlation of trends based on the ratio may be reversed.

The term "recombinant host cell" (or simply "host cell") refers to a cell that comprises a nucleic acid that is not naturally present in the cell, such as a cell into which a recombinant expression vector has been introduced. It should be understood that cells according to the present invention is intended to refer not only to the particular subject cell, but also encompasses progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term cell according to the present invention.

The term "cancer response," "response to immunotherapy," or "response to modulators of T-cell mediated cytotoxicity/immunotherapy combination therapy" relates to any response of the hyperproliferative disorder (*e.g.*, cancer) to a cancer agent, such as a modulator of T-cell mediated cytotoxicity, and an immunotherapy, preferably to a change in tumor mass and/or volume after initiation of neoadjuvant or adjuvant therapy. The term "neoadjuvant therapy" refers to a treatment given before the primary treatment. Examples of neoadjuvant therapy may include chemotherapy, radiation therapy, and hormone therapy. Hyperproliferative disorder response may be assessed, for example for efficacy or in a neoadjuvant or adjuvant situation, where the size of a tumor after systemic intervention may be compared to the initial size and dimensions as measured by CT, PET, mammogram, ultrasound or palpation. Responses may also be assessed by caliper measurement or

pathological examination of the tumor after biopsy or surgical resection. Response may be recorded in a quantitative fashion like percentage change in tumor volume or in a qualitative fashion like "pathological complete response" (pCR), "clinical complete remission" (cCR), "clinical partial remission" (cPR), "clinical stable disease" (cSD), "clinical progressive disease" (cPD) or other qualitative criteria. Assessment of hyperproliferative disorder response may be done early after the onset of neoadjuvant or adjuvant therapy, *e.g.*, after a few hours, days, weeks or preferably after a few months. A typical endpoint for response assessment is upon termination of neoadjuvant chemotherapy or upon surgical removal of residual tumor cells and/or the tumor bed. This is typically three months after initiation of neoadjuvant therapy. In some embodiments, clinical efficacy of the therapeutic treatments described herein may be determined by measuring the clinical benefit rate (CBR). The clinical benefit rate is measured by determining the sum of the percentage of patients who are in complete remission (CR), the number of patients who are in partial remission (PR) and the number of patients having stable disease (SD) at a time point at least 6 months out from the end of therapy. The shorthand for this formula is CBR=CR+PR+SD over 6 months. In some embodiments, the CBR for a particular cancer therapeutic regimen is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or more. Additional criteria for evaluating the response to cancer therapies are related to "survival," which includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said survival may be calculated by reference to a defined start point (*e.g.*, time of diagnosis or start of treatment) and end point (*e.g.*, death, recurrence or metastasis). In addition, criteria for efficacy of treatment may be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence. For example, in order to determine appropriate threshold values, a particular cancer therapeutic regimen may be administered to a population of subjects and the outcome may be correlated to biomarker measurements that were determined prior to administration of any cancer therapy. The outcome measurement may be pathologic response to therapy given in the neoadjuvant setting. Alternatively, outcome measures, such as overall survival and disease-free survival may be monitored over a period of time for subjects following cancer therapy for which biomarker measurement values are known. In certain embodiments, the doses administered are standard doses known

in the art for cancer therapeutic agents. The period of time for which subjects are monitored may vary. For example, subjects may be monitored for at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, or 60 months. Biomarker measurement threshold values that correlate to outcome of a cancer therapy may be determined using well-known methods in the art, such as those described in the Examples section.

As indicated, the terms may also refer to an improved prognosis, for example, as reflected by an increased time to recurrence, which is the period to first recurrence censoring for second primary cancer as a first event or death without evidence of recurrence, or an increased overall survival, which is the period from treatment to death from any cause. To respond or to have a response means there is a beneficial endpoint attained when exposed to a stimulus. Alternatively, a negative or detrimental symptom is minimized, mitigated or attenuated on exposure to a stimulus. It will be appreciated that evaluating the likelihood that a tumor or subject will exhibit a favorable response is equivalent to evaluating the likelihood that the tumor or subject will not exhibit favorable response (*i.e.*, will exhibit a lack of response or be non-responsive).

The term "resistance" refers to an acquired or natural resistance of a cancer sample or a mammal to a cancer therapy (*i.e.*, being nonresponsive to or having reduced or limited response to the therapeutic treatment), such as having a reduced response to a therapeutic treatment by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or more, such 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold or more, or any range in between, inclusive. The reduction in response may be measured by comparing with the same cancer sample or mammal before the resistance is acquired, or by comparing with a different cancer sample or a mammal that is known to have no resistance to the therapeutic treatment. A typical acquired resistance to chemotherapy is called "multidrug resistance." The multidrug resistance may be mediated by P-glycoprotein or may be mediated by other mechanisms, or it may occur when a mammal is infected with a multi-drug-resistant microorganism or a combination of microorganisms. The determination of resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician, for example, may be measured by cell proliferative assays and cell death assays as described herein as "sensitizing." In some embodiments, the term "reverses resistance" means that the use of a second agent in combination with a primary cancer therapy (*e.g.*, chemotherapeutic or radiation therapy) is able to produce a significant decrease in tumor volume at a level of statistical significance (*e.g.*, $p<0.05$) when compared to tumor volume of untreated tumor in the circumstance where the primary cancer therapy

(*e.g.*, chemotherapeutic or radiation therapy) alone is unable to produce a statistically significant decrease in tumor volume compared to tumor volume of untreated tumor. This generally applies to tumor volume measurements made at a time when the untreated tumor is growing logarithmically.

The term "sample" used for detecting or determining the absence, presence, or level of at least one biomarker is typically brain tissue, cerebrospinal fluid, whole blood, plasma, serum, saliva, urine, stool (*e.g.*, feces), tears, and any other bodily fluid (*e.g.*, as described above under the definition of "body fluids"), or a tissue sample (*e.g.*, biopsy) such as a skin, colon sample, or surgical resection tissue. In some embodiments, methods encompassed by the present invention further comprises obtaining the sample from the individual prior to detecting or determining the absence, presence, or level of at least one marker in the sample.

The term "sensitize" means to alter cancer cells or tumor cells in a way that allows for more effective treatment of the associated cancer with a cancer therapy (*e.g.*, anti-immune checkpoint, chemotherapeutic, and/or radiation therapy). In some embodiments, normal cells are not affected to an extent that causes the normal cells to be unduly injured by the therapies. An increased sensitivity or a reduced sensitivity to a therapeutic treatment is measured according to a known method in the art for the particular treatment and methods described herein below, including, but not limited to, cell proliferative assays (Tanigawa *et al.* (1982) *Cancer Res.* 42:2159-2164) and cell death assays (Weisenthal *et al.* (1984) *Cancer Res.* 94:161-173; Weisenthal *et al.* (1985) *Cancer Treat Rep.* 69:615-632; Weisenthal *et al.*, In: Kaspers G J L, Pieters R, Twentyman P R, Weisenthal L M, Veerman A J P, eds. Drug Resistance in Leukemia and Lymphoma. Langhorne, P A: Harwood Academic Publishers, 1993:415-432; Weisenthal (1994) *Contrib. Gynecol. Obstet.* 19:82-90). The sensitivity or resistance may also be measured in animal by measuring the tumor size reduction over a period of time, for example, 6 month for human and 4-6 weeks for mouse. A composition or a method sensitizes response to a therapeutic treatment if the increase in treatment sensitivity or the reduction in resistance is 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, or more, such 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold or more, or any range in between, inclusive, compared to treatment sensitivity or resistance in the absence of such composition or method. The determination of sensitivity or resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician. It is to be understood that any method described herein for enhancing the efficacy of a cancer therapy may be equally applied to

methods for sensitizing hyperproliferative or otherwise cancerous cells (*e.g.*, resistant cells) to the cancer therapy.

The term "small molecule" is a term of the art and includes molecules that are less than about 1000 molecular weight or less than about 500 molecular weight. In one embodiment, small molecules do not exclusively comprise peptide bonds. In another embodiment, small molecules are not oligomeric. Exemplary small molecule compounds which may be screened for activity include, but are not limited to, peptides, peptidomimetics, nucleic acids, carbohydrates, small organic molecules (*e.g.*, polyketides) (Cane *et al.* (1998) *Science* 282:63-68), and natural product extract libraries. In another embodiment, the compounds are small, organic non-peptidic compounds. In a further embodiment, a small molecule is not biosynthetic.

The term "specific binding" refers to binding protein binding to a predetermined antigen. Typically, the binding protein binds with an affinity (K_D) of approximately less than or equal to about $5x10^{-4}$ M, less than or equal to about $1x10^{-4}$ M, less than or equal to about $5x10^{-5}$ M, less than or equal to about $1x10^{-5}$ M, less than or equal to about $5x10^{-6}$ M, less than or equal to about $1x10^{-6}$ M, less than or equal to about $5x10^{-7}$ M, less than or equal to about $1x10^{-7}$ M, less than or equal to about $5x10^{-8}$ M, less than or equal to about $1x10^{-8}$ M, less than or equal to about $5x10^{-9}$ M, less than or equal to about $1x10^{-9}$ M, less than or equal to about $5x10^{-10}$ M, less than or equal to about $1x10^{-10}$ M, less than or equal to about $5x10^{-11}$ M, less than or equal to about $1x10^{-11}$ M, less than or equal to about $5x10^{-12}$ M, less than or equal to about $1x10^{-12}$ M, or even lower, or any range in between, inclusive, such as between about 1-50 micromolar, 1-100 micromolar, 0.1-500 micromolar, and the like,when determined by a binding assay, such as surface plasmon resonance (SPR) technology in a BIAcore™ assay instrument using an antigen of interest as the analyte and the binding protein as the ligand. In some embodiments, the binding protein binds to the predetermined antigen with an affinity that is at least 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, 4.5-, 5.0-, 6.0-, 7.0-, 8.0-, 9.0-, or 10.0-fold or greater than its affinity for binding to a non-specific antigen (*e.g.*, BSA, casein) other than the predetermined antigen or a closely-related antigen. The phrases "a binding protein recognizing an antigen" and "a binding protein specific for an antigen" are used interchangeably herein with the term "a binding protein which binds specifically to an antigen." Selective binding is a relative term referring to the ability of a binding protein to discriminate the binding of one antigen over another, such as a particular family member or antigen target over a related family member or antigen target. For example, analytical data provided in the Examples section demonstrates that binding proteins

described herein specifically bind MAGEC2 immunogenic epitopes and/or selectively bind a number of related epitopes (*e.g.*, MAGEC2 immunogenic epitopes and closely related sequences) discriminating such targets from the vast majority of other possible epitopes available in the human genome.

The term "subject" refers to any healthy animal, mammal or human, or any animal, mammal or human afflicted with a disorder characterized by MAGEC2 expression, such as a non-malignant disorder, a hyperproliferative disorder, or a relapse of ahyperpoliferative disorder characterized by MAGEC2 expression. The term "subject" is interchangeable with "patient."

The term "survival" includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said survival may be calculated by reference to a defined start point (*e.g.*, time of diagnosis or start of treatment) and end point (*e.g.*, death, recurrence or metastasis). In addition, criteria for efficacy of treatment may be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence.

The term "synergistic effect" refers to the combined effect of two or more agents (*e.g.*, a MAGEC2-related agent described herein and another therapy, such as an additional MAGEC2-targeted TCR, anti-cancer therapy, immunotherapy, etc., for treating a disorder characterized by MAGEC2 expression) that is greater than the sum of the separate effects of the cancer agents/therapies alone.

As used herein, the term "T cell-mediated response" refers to a response mediated by T cells, including effector T cells (*e.g.*, $CD8^+$ cells) and helper T cells (*e.g.*, $CD4^+$ cells). T cell mediated responses include, for example, T cell cytotoxicity and proliferation.

A "transcribed polynucleotide" or "nucleotide transcript" is a polynucleotide (*e.g.*, an mRNA, hnRNA, a cDNA, or an analog of such RNA or cDNA) which is complementary to or homologous with all or a portion of a mature mRNA made by transcription of a biomarker nucleic acid and normal post-transcriptional processing (*e.g.*, splicing), if any, of the RNA transcript, and reverse transcription of the RNA transcript.

A "T cell" is an immune system cell that matures in the thymus and produces T cell receptors (TCRs). T cells may be naive (not exposed to antigen; increased expression of

CD62L, CCR7, CD28, CD3, CD 127, 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 may 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 naive T cells) and effector memory T cells (T_{EM}, decreased expression of CD62L, CCR7, CD28, CD45RA, and increased expression of CD127 as compared to naive T cells or T_{CM}). Effector T cells (T_E) refers to antigen-experienced CD8+ cytotoxic T lymphocytes that have decreased expression of CD62L ,CCR7, CD28, and are positive for granzyme and perforin as compared to T_{CM}. Other exemplary T cells include regulatory T cells, such as $CD4^+$ $CD25^+$ ($Foxp3^+$) regulatory T cells and Tregl7 cells, as well as Trl, Th3, $CD8^+$CD28, and Qa-1 restricted T cells.

Conventional T cells, also known as Tconv or Teffs, have effector functions (*e.g.*, cytokine secretion, cytotoxic activity, anti-self-recognition, and the like) to increase immune responses by virtue of their expression of one or more T cell receptors. Tcons or Teffs are generally defined as any T cell population that is not a Treg and include, for example, naïve T cells, activated T cells, memory T cells, resting Tcons, or Tcons that have differentiated toward, for example, the Th1 or Th2 lineages. In some embodiments, Teffs are a subset of non-Treg T cells. In some embodiments, Teffs are CD4+ Teffs or CD8+ Teffs, such as CD4+ helper T lymphocytes (*e.g.*, Th0, Th1, Tfh, or Th17) and CD8+ cytotoxic T lymphocytes. As described further herein, cytotoxic T cells are CD8+ T lymphocytes. "Naïve Tcons" are $CD4^+$ T cells that have differentiated in bone marrow, and successfully underwent a positive and negative processes of central selection in a thymus, but have not yet been activated by exposure to an antigen. Naïve Tcons are commonly characterized by surface expression of L-selectin (CD62L), absence of activation markers such as CD25, CD44 or CD69, and absence of memory markers such as CD45RO. Naïve Tcons are therefore believed to be quiescent and non-dividing, requiring interleukin-7 (IL-7) and interleukin-15 (IL- 15) for homeostatic survival (see, at least WO 2010/101870). The presence and activity of such cells are undesired in the context of suppressing immune responses. Unlike Tregs, Tcons are not anergic and can proliferate in response to antigen-based T cell receptor activation (Lechler *et al.* (2001) *Philos. Trans. R. Soc. Lond. Biol. Sci.* 356:625-637).

"T effector" ("T_{eff}" or "T_E") cells refers to T cells (*e.g.*, CD4+ and CD8+ T cells) with cytolytic activities as well as T helper (Th) cells, which secrete cytokines and activate and direct other immune cells, but does not include regulatory T cells (Treg cells).

"T cell receptor" or "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.* (1997) *Curr. Biol. Publ.* 4:33) that is capable of binding (*e.g., e.g.*, specifically and/or selectively) to an antigen peptide bound to an 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 alpha and beta chains (also known as TCRα and TCRβ, respectively), or γ and δ chains (also known as TCRγ and TCRδ, respectively). Like immunoglobulins (*e.g.,* antibodies), the extracellular portion of TCR chains (*e.g.,* α-chain and β-chain) contain two immunoglobulin domains: a variable domain (*e.g.,* α-chain variable domain or V_α and β-chain variable domain or V_β; typically amino acids 1 to 116 based on Kabat numbering (Kabat *et al.* (1991) "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 5th ed.) at the N-terminal end, 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) at the C-terminal end and adjacent to the cell membrane. Also like immunoglobulins, the variable domains contain complementary determining regions ("CDRs", also called hypervariable regions or "HVRs") separated by framework regions ("FRs") (*see, e.g.,* Fores *et al.* (1990) *Proc. Natl. Acad Sci. US.A.* 87:9138; Chothia *et al.* (1988) *EMBO J.* 7:3745; Lefranc *et al.* (2003) *Dev. Comp. Immunol.* 27:55). In some embodiments, a TCR is found on the surface of a T cell (or T lymphocyte) and associates with the CD3 complex. The source of a TCR encompassed by the present invention may be from various animal species, such as a human, mouse, rat, rabbit or other mammal.

The term "T cell receptor" or "TCR" should be understood to encompass full TCRs as well as antigen-binding portions or antigen-binding fragments thereof. In some embodiments, the TCR is an intact or full-length TCR, including TCRs in the αβ form or γδ form. In some embodiments, the TCR is an antigen-binding portion that is less than a full-length TCR but that binds to a specific peptide bound in an MHC molecule, such as binds to an MHC-peptide complex. In some cases, an antigen-binding portion or fragment of a TCR may contain only a portion of the structural domains of a full-length or intact TCR, but yet is able to bind the peptide epitope, such as MHC-peptide complex, to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable α chain and variable β chain of a TCR, sufficient to form a binding site for binding

to a specific MHC-peptide complex. Generally, the variable chains of a TCR contain complementarity determining regions (CDRs) involved in recognition of the peptide, MHC and/or MHC-peptide complex.

Nomenclature established by the International Immunogenetics Information System (IMGT) (see also Scaviner and Lefranc (2000) *Exp. Clin. Immunogenet.* 17:83-96 and 97-106; Folch and Lefranc (2000) *Exp. Clin. Immunogenet,* 17:107-114; T Cell Receptor Factsbook", (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8). The IMGT provides unique sequences used to describe a TCR, and sequences described herein may be identified by reference to such unique sequences provided herein. TCR sequences are publicly available at the IMGT database at imgt.org.

As described above, native alpha/beta heterodimeric TCRs have an alpha chain and a beta chain. Broadly, each chain comprises variable, joining and constant regions, and the beta chain also usually contains a short diversity region between the variable and joining regions, but this diversity region is often considered as part of the joining region. Each variable region comprises three hypervariable CDRs (Complementarity Determining Regions) embedded in a framework sequence. CDR3 is well-known to be the main mediator of antigen recognition. There are several types of alpha chain variable (Vα) regions and several types of beta chain variable (Vβ) regions distinguished by their framework, CDR1 and CDR2 sequences, and by a partly defined CDR3 sequence. The Vα types are referred to in IMGT nomenclature by a unique TRAV number. For example, "TRAV4" defines a TCR Vα region having unique framework and CDR1 and CDR2 sequences, and a CDR3 sequence which is partly defined by an amino acid sequence which is preserved from TCR to TCR but which also includes an amino acid sequence which varies from TCR to TCR. Similarly, "TRBV2" defines a TCR Vβ region having unique framework and CDR1 and CDR2 sequences, but with only a partly defined CDR3 sequence. It is known that there are 54 alpha variable genes, of which 44 are functional, and 67 beta variable genes, of which 42 are functional, within the alpha and beta loci, respectively.

The joining regions of the TCR are similarly defined by the unique IMGT TRAJ and TRBJ nomenclature, and the constant regions by the IMGT TRAC and TRBC nomenclature. The beta chain diversity region is referred to in IMGT nomenclature by the abbreviation TRBD, and, as mentioned, the concatenated TRBD/TRBJ regions are often considered together as the joining region.

The gene pools that encode the TCR alpha and beta chains are located on different chromosomes and contain separate V, (D), J and C gene segments, which are brought

together by rearrangement during T cell development. This leads to a very high diversity of T cell alpha and beta chains due to the large number of potential recombination events that occur between the 54 TCR alpha variable genes and 61 alpha J genes or between the 67 beta variable genes, two beta D genes and 13 beta J genes. The recombination process is not precise and introduces further diversity within the CDR3 region. Each alpha and beta variable gene may also comprise allelic variants, designated in IMGT nomenclature as TRAVxx*01 and *02, or TRBVx-x*01 and *02 respectively, thus further increasing the amount of variation. In the same way, some of the TRBJ sequences have two known variations. (Note that the absence of a "*" qualifier means that only one allele is known for the relevant sequence). The natural repertoire of human TCRs resulting from recombination and thymic selection has been estimated to comprise approximately 10^6 unique beta chain sequences, determined from CDR3 diversity (Arstila *et al.* (1999) *Science* 286:958-961) and could be even higher (Robins *et al.* (2009) *Blood* 114:4099-4107). Each beta chain is estimated to pair with at least 25 different alpha chains, thus generating further diversity (Arstila *et al.* (1999) Science 286:958-961).

The term "TCR alpha variable domain" therefore refers to the concatenation of TRAV and TRAJ regions; a TRAV region only; or TRAV and a partial TRAJ region, and the term TCR alpha constant domain refers to the extracellular TRAC region, or to a C-terminal truncated or full length TRAC sequence. Likewise the term "TCR beta variable domain" refers to the concatenation of TRBV and TRBD/TRBJ regions; to the TRBV and TRBD regions only; to the TRBV and TRBJ regions only; or to the TRBV and partial TRBD and/or TRBJ regions, and the term TCR beta constant domain refers to the extracellular TRBC region, or to a C-terminal truncated or full length TRBC sequence. These TCR alpha variable domain and TCR beta variable domain nomenclature similarly applies to the variable domains of TCR gamma and TCR delta chains, respectively, for gamma/delta TCRs. An ordinarily skilled artisan can obtain TRAV, TRAJ, TRAC, TRBV, TRBJ, and TRBC gene sequences, such as through the publicly available IMGT database.

The term "TCR complex" refers to a complex formed by the association of CD3 with TCR. For example, a TCR complex may 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 may be composed of a CD3γ chain, a CD3δ chain, two CD3ε chains, a homodimer of CD3ζ chains, a TCRγ chain, and a TCRδ chain.

The term "therapeutic effect" refers to a local or systemic effect in animals, particularly mammals, and more particularly humans, caused by a pharmacologically active substance. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and conditions in an animal or human.

The terms "therapeutically effective amount" and "effective amount" means that amount of a substance that produces some desired effect, such as a desired local or systemic therapeutic effect, in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any treatment. In some embodiments, a therapeutically effective amount of a substance will depend on the substance's therapeutic index, solubility, pharmacokinetics, half-life, and the like. Toxicity and therapeutic efficacy of subject compounds may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} and the ED_{50}. In some embodiments, compositions that exhibit large therapeutic indices are used. In some embodiments, the LD_{50} (lethal dosage) may be measured and may be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more reduced for the agent relative to no administration of the agent. Similarly, the ED_{50} (*i.e.*, the concentration which achieves a half-maximal inhibition of symptoms) may be measured and may be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the agent relative to no administration of the agent. Also, similarly, the IC_{50} may be measured and may be, for example, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000% or more increased for the agent relative to no administration of the agent. In some embodiments, T cell immune response in an assay may be increased by at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100%. In another embodiment, at least about a 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% decrease in a viral load may be achieved.

The term "treat" refers to the therapeutic management or improvement of a condition (*e.g.*, a disease or disorder) of interest. Treatment may include, but is not limited to, administering an agent or composition (*e.g.*, a pharmaceutical composition) to a subject. Treatment is typically undertaken in an effort to alter the course of a disease (which term is used to indicate any disease, disorder, syndrome or undesirable condition warranting or

potentially warranting therapy) in a manner beneficial to the subject. The effect of treatment may include reversing, alleviating, reducing severity of, delaying the onset of, curing, inhibiting the progression of, and/or reducing the likelihood of occurrence or recurrence of the disease or one or more symptoms or manifestations of the disease. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. A therapeutic agent may be administered to a subject who has a disease or is at increased risk of developing a disease relative to a member of the general population. In some embodiments, a therapeutic agent may be administered to a subject who has had a disease but no longer shows evidence of the disease. The agent may be administered *e.g.*, to reduce the likelihood of recurrence of evident disease. A therapeutic agent may be administered prophylactically, *i.e.*, before development of any symptom or manifestation of a disease. "Prophylactic treatment" refers to providing medical and/or surgical management to a subject who has not developed a disease or does not show evidence of a disease in order, *e.g.*, to reduce the likelihood that the disease will occur or to reduce the severity of the disease should it occur. The subject may have been identified as being at risk of developing the disease (*e.g.*, at increased risk relative to the general population or as having a risk factor that increases the likelihood of developing the disease.

The term "unresponsiveness" includes refractivity of cancer cells to therapy or refractivity of therapeutic cells, such as immune cells, to stimulation, *e.g.*, stimulation via an activating receptor or a cytokine. Unresponsiveness may occur, *e.g.*, because of exposure to immunosuppressants or exposure to high doses of antigen. As used herein, the term "anergy" or "tolerance" includes refractivity to activating receptor-mediated stimulation. Such refractivity is generally antigen-specific and persists after exposure to the tolerizing antigen has ceased. For example, anergy in T cells (as opposed to unresponsiveness) is characterized by lack of cytokine production, *e.g.*, IL-2. T cell anergy occurs when T cells are exposed to antigen and receive a first signal (a T cell receptor or CD-3 mediated signal) in the absence of a second signal (a costimulatory signal). Under these conditions, reexposure of the cells to the same antigen (even if reexposure occurs in the presence of a costimulatory polypeptide) results in failure to produce cytokines and, thus, failure to proliferate. Anergic T cells may, however, proliferate if cultured with cytokines (*e.g.*, IL-2). For example, T cell anergy may also be observed by the lack of IL-2 production by T lymphocytes as measured by ELISA or

by a proliferation assay using an indicator cell line. Alternatively, a reporter gene construct may be used. For example, anergic T cells fail to initiate IL-2 gene transcription induced by a heterologous promoter under the control of the 5' IL-2 gene enhancer or by a multimer of the AP1 sequence that may be found within the enhancer (Kang *et al.* (1992) *Science* 257:1134).

The term "vaccine" refers to a pharmaceutical composition that elicits an immune response to an antigen of interest. The vaccine may also confer protective immunity upon a subject.

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

The term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. In some embodiments, a vector is an episome, *i.e.*, a nucleic acid capable of extra-chromosomal replication. In some embodiments, vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer generally to circular double stranded DNA loops, which, in their vector form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, as will be appreciated by those skilled in the art, the present invention is intended to include such other forms of expression vectors that serve equivalent functions and which become subsequently known in the art.

There is a known and definite correspondence between the amino acid sequence of a particular protein and the nucleotide sequences that can code for the protein, as defined by the genetic code (shown below). Likewise, there is a known and definite correspondence between the nucleotide sequence of a particular nucleic acid and the amino acid sequence encoded by that nucleic acid, as defined by the genetic code.

GENETIC CODE

Alanine (Ala, A)	GCA, GCC, GCG, GCT
Arginine (Arg, R)	AGA, ACG, CGA, CGC, CGG, CGT
Asparagine (Asn, N)	AAC, AAT
Aspartic acid (Asp, D)	GAC, GAT
Cysteine (Cys, C)	TGC, TGT
Glutamic acid (Glu, E)	GAA, GAG
Glutamine (Gln, Q)	CAA, CAG
Glycine (Gly, G)	GGA, GGC, GGG, GGT
Histidine (His, H)	CAC, CAT
Isoleucine (Ile, I)	ATA, ATC, ATT
Leucine (Leu, L)	CTA, CTC, CTG, CTT, TTA, TTG
Lysine (Lys, K)	AAA, AAG
Methionine (Met, M)	ATG
Phenylalanine (Phe, F)	TTC, TTT
Proline (Pro, P)	CCA, CCC, CCG, CCT
Serine (Ser, S)	AGC, AGT, TCA, TCC, TCG, TCT
Threonine (Thr, T)	ACA, ACC, ACG, ACT
Tryptophan (Trp, W)	TGG
Tyrosine (Tyr, Y)	TAC, TAT
Valine (Val, V)	GTA, GTC, GTG, GTT
Termination signal (end)	TAA, TAG, TGA

An important and well-known feature of the genetic code is its redundancy, whereby, for most of the amino acids used to make proteins, more than one coding nucleotide triplet may be employed (illustrated above). Therefore, a number of different nucleotide sequences may code for a given amino acid sequence. Such nucleotide sequences are considered functionally equivalent since they result in the production of the same amino acid sequence in

all organisms (although certain organisms may translate some sequences more efficiently than they do others). Moreover, occasionally, a methylated variant of a purine or pyrimidine may be found in a given nucleotide sequence. Such methylations do not affect the coding relationship between the trinucleotide codon and the corresponding amino acid.

In view of the foregoing, the nucleotide sequence of a DNA or RNA encoding a biomarker nucleic acid (or any portion thereof) may be used to derive the polypeptide amino acid sequence, using the genetic code to translate the DNA or RNA into an amino acid sequence. Likewise, for polypeptide amino acid sequence, corresponding nucleotide sequences that can encode the polypeptide can be deduced from the genetic code (which, because of its redundancy, will produce multiple nucleic acid sequences for any given amino acid sequence). Thus, description and/or disclosure herein of a nucleotide sequence which encodes a polypeptide should be considered to also include description and/or disclosure of the amino acid sequence encoded by the nucleotide sequence. Similarly, description and/or disclosure of a polypeptide amino acid sequence herein should be considered to also include description and/or disclosure of all possible nucleotide sequences that can encode the amino acid sequence.

II. Peptides

In certain aspects, provided herein are methods and compositions for the treatment and/or prevention of disorders associated with MAGEC2 expression through the induction of an immune response against MAGEC2 or cells expressing MAGEC2 relating to administration of MAGEC2 immunogenic peptides, nucleic acids encoding same, and/or cells expressing same, described herein.

In certain embodiments, the MAGEC2 immunogenic peptide comprises (*e.g.*, consists of) a peptide epitope selected from peptide sequences listed in Table 1. Peptide epitopes described herein may be combined with MHC molecules, such as particular HLA molecules having particular HLA alpha chain alleles. For example, peptides listed in Table 1 were identified in association with an MHC whose alpha chain had an HLA-B*07 serotype, such as that encoded by an HLA-B*0702 allele, as described further in the Examples section. In some embodiments, MAGEC2 immunogenic peptides may be combined with an MHC molecule, wherein the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702. In some embodiments, the MAGEC2 immunogenic peptides are derived from a human MAGEC2

protein and/or a MAGEC2 protein shown in Table 3. In some embodiments, one or more MAGEC2 immunogenic peptides are administered alone or in combination with an adjuvant.

In certain aspects, provided herein are compositions comprising one or more MAGEC2 immunogenic peptides described herein and an adjuvant.

Table 1: MAGEC2 epitopes

MAGEC2 epitopes presented by HLA serotype HLA-B*07

Peptide Epitopes
RAREFMEL
RAREFMELL
RAREFMELLF
LKRAREFMEL
VILKRAREF
FPVILKRAR
KRAREFMEL
KRAREFMELL
LKRAREFMELL
RAREFMELLFG

* Included in Table 1, are peptide epitopes, as well as polypeptide molecules comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with an amino acid sequence of any sequence listed in Table 1, or a portion thereof. Such polypeptides may have a function of the full-length peptide or polypeptide as described further herein.

In some embodiments, provided herein are MAGEC2 polypeptides and/or nucleic acids encoding MAGEC2 polypeptides. In some embodiments, MAGEC2 polypeptides are polypeptides that include an amino acid sequence of sufficient length to elicit a MAGEC2-specific immune response. In certain embodiments, the MAGEC2 polypeptide also includes amino acids that do not correspond to the amino acid sequence (*e.g.*, a fusion protein comprising a MAGEC2 amino acid sequence and an amino acid sequence corresponding to a non- MAGEC2 protein or polypeptide). In some embodiments, the MAGEC2 polypeptide only includes amino acid sequence corresponding to a MAGEC2 protein or fragment thereof.

In some embodiments, the MAGEC2 polypeptide has an amino acid sequence that comprises, consists essentially of, or consists of at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17,

18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 373, or more, or any range in between inclusive (*e.g.*, 7-25, 8-22, 9-22, etc.) consecutive amino acids of a MAGEC2 protein amino acid sequence, such as those set forth in Table 3. In some embodiments, the consecutive amino acids are identical to an amino acid sequence of MAGEC2 set forth in Table 3. In some embodiments, MAGEC2 polypeptides comprise, consist essentially of, or consist of one or more peptide epitopes selected from the group consisting of MAGEC2 peptide epitopes listed in Table 1.

As is well-known to those skilled in the art, polypeptides having substantial sequence similarities can cause identical or very similar immune reaction in a host animal. Accordingly, in some embodiments, a derivative, equivalent, variant, fragment, or mutant of a MAGEC2 immunogenic peptide described herein or fragment thereof may also suitable for the methods and compositions provided herein.

In some embodiments, variations or derivatives of the MAGEC2 immunogenic polypeptides are provided herein. The altered polypeptide may have an altered amino acid sequence, for example by conservative substitution, yet still elicits immune responses which react with the unaltered protein antigen, and are considered functional equivalents. As used herein, the term "conservative substitution" denotes the replacement of an amino acid residue by another, biologically similar residue. It is well-known in the art that the amino acids within the same conservative group may typically substitute for one another without substantially affecting the function of a protein. According to certain embodiments, the derivative, equivalents, variants, or mutants of the ligand-binding domain of a MAGEC2 immunogenic peptide are polypeptides that are at least 85% homologous to the sequence of a MAGEC2 immunogenic peptide described herein or fragment thereof. In some embodiments, the homology is at least 90%, at least 95%, at least 98%, or more.

Immunogenic peptides encompassed by the present invention may comprise a peptide epitope derived from a MAGEC2 protein, such as those listed in Table 1. In some embodiments, the immunogenic peptide is 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length. In some embodiments, the peptide amino acid sequences is modified, which may include conservative or non-conservative mutations. A peptide may comprise at most 1, 2, 3,

4, or more mutations. In some embodiments, a peptide may comprise at least 1, 2, 3, 4, or more mutations.

In some embodiments, a peptide may be chemically modified. For example, a peptide can be mutated to modify peptide properties such as detectability, stability, biodistribution, pharmacokinetics, half-life, surface charge, hydrophobicity, conjugation sites, pH, function, and the like. N-methylation is one example of methylation that can occur in a peptide of the disclosure. In some embodiments, a peptide may be modified by methylation on free amines such as by reductive methylation with formaldehyde and sodium cyanoborohydride.

A chemical modification may comprise a polymer, a polyether, polyethylene glycol, a biopolymer, a zwitterionic polymer, a polyamino acid, a fatty acid, a dendrimer, an Fc region, a simple saturated carbon chain such as palmitate or myristolate, or albumin. The chemical modification of a peptide with an Fc region may be a fusion Fc-peptide. A polyamino acid may include, for example, a poly amino acid sequence with repeated single amino acids (*e.g.*, poly glycine), and a poly amino acid sequence with mixed poly amino acid sequences that may or may not follow a pattern, or any combination of the foregoing. In some embodiments, the peptides encompassed by the present disclosure may be modified such that the modification increases the stability and/or the half-life of the peptides. In some embodiments, the attachment of a hydrophobic moiety, such as to the N-terminus, the C-terminus, or an internal amino acid, can be used to extend half-life of a peptide encompassed by the present disclosure. In other embodiments, a peptide may include post-translational modifications (*e.g.*, methylation and/or amidation), which can affect, for example, serum half-life. In some embodiments, simple carbon chains (*e.g.*, by myristoylation and/or palmitylation) can be conjugated to the fusion proteins or peptides. In some embodiments, the simple carbon chains may render the fusion proteins or peptides easily separable from the unconjugated material. For example, methods that may be used to separate the fusion proteins or peptides from the unconjugated material include, but are not limited to, solvent extraction and reverse phase chromatography. The lipophilic moieties can extend half-life through reversible binding to serum albumin. The conjugated moieties may be lipophilic moieties that extend half-life of the peptides through reversible binding to serum albumin. In some embodiments, the lipophilic moiety may be cholesterol or a cholesterol derivative, including cholestenes, cholestanes, cholestadienes and oxysterols. In some embodiments, the peptides may be conjugated to myristic acid (tetradecanoic acid) or a derivative thereof. In other embodiments, a peptide may be coupled (*e.g.*, conjugated) to a half-life modifying agent. Examples of half-life modifying agents include but are not limited to: a polymer, a

polyethylene glycol (PEG), a hydroxyethyl starch, polyvinyl alcohol, a water soluble polymer, a zwitterionic water soluble polymer, a water soluble poly(amino acid), a water soluble polymer of proline, alanine and serine, a water soluble polymer containing glycine, glutamic acid, and serine, an Fc region, a fatty acid, palmitic acid, or a molecule that binds to albumin. In some embodiments, a spacer or linker may be coupled to a peptide, such as 1, 2, 3, 4, or more amino acid residues that serve as a spacer or linker in order to facilitate conjugation or fusion to another molecule, as well as to facilitate cleavage of the peptide from such conjugated or fused molecules. In some embodiments, fusion proteins or peptides may be conjugated to other moieties that, for example, can modify or effect changes to the properties of the peptides.

A peptide may, in some embodiments, be covalently linked to a moiety. In some embodiments, the covalently linked moiety comprises an affinity tag or a label. The affinity tag may be selected from the group consisting of glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, HaloTag, HA tag, Flag® tag, His tag, biotin tag, and V5 tag. The label may be a fluorescent protein. In some embodiments, the covalently linked moiety is selected from the group consisting of an inflammatory agent, an anti-inflammatory agent, a cytokine, a toxin, a cytotoxic molecule, a radioactive isotope, or an antibody such as a single-chain Fv.

A peptide may be conjugated to an agent used in imaging, research, therapeutics, theranostics, pharmaceuticals, chemotherapy, chelation therapy, targeted drug delivery, and radiotherapy. In some embodiments, a peptide may be conjugated to or fused with detectable agents, such as a fluorophore, a near-infrared dye, a contrast agent, a nanoparticle, a metal-containing nanoparticle, a metal chelate, an X-ray contrast agent, a PET agent, a metal, a radioisotope, a dye, radionuclide chelator, or another suitable material that can be used in imaging. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more detectable moieties may be linked to a peptide. Non-limiting examples of radioisotopes include alpha emitters, beta emitters, positron emitters, and gamma emitters. In some embodiments, the metal or radioisotope is selected from the group consisting of actinium, americium, bismuth, cadmium, cesium, cobalt, europium, gadolinium, iridium, lead, lutetium, manganese, palladium, polonium, radium, ruthenium, samarium, strontium, technetium, thallium, and yttrium. In some embodiments, the metal is actinium, bismuth, lead, radium, strontium, samarium, or yttrium. In some embodiments, the radioisotope is actinium-225 or lead-212. In some embodiments, the near-infrared dyes are not easily quenched by biological tissues and fluids. In some embodiments, the fluorophore is a fluorescent agent emitting

electromagnetic radiation at a wavelength between 650 nm and 4000 nm, such emissions being used to detect such agent. Non-limiting examples of fluorescent dyes that may be used as a conjugating molecule include DyLight®-680, DyLight®-750, VivoTag®-750, DyLight®-800, IRDye®-800, VivoTag®-680, Cy5.5, ZQ800, or indocyanine green (ICG). In some embodiments, near infrared dyes often include cyanine dyes (*e.g.*, Cy7, Cy5.5, and Cy5). Additional non-limiting examples of fluorescent dyes for use as a conjugating molecule in the present disclosure include acradine orange or yellow, Alexa Fluors® (*e.g.*, Alexa Fluor® 790, 750, 700, 680, 660, and 647) and any derivative thereof, 7-actinomycin D, 8-anilinonaphthalene-1-sulfonic acid, ATTO dye and any derivative thereof, auramine-rhodamine stain and any derivative thereof, bensantrhone, bimane, 9-10-bis(phenylethynyl)anthracene, 5,12-bis(phenylethynyl)naththacene, bisbenzimide, brainbow, calcein, carbodyfluorescein and any derivative thereof, 1-chloro-9,10-bis(phenylethynyl)anthracene and any derivative thereof, DAPI, DiOC6, DyLight Fluors and any derivative thereof, epicocconone, ethidium bromide, FlAsH-EDT2, Fluo dye and any derivative thereof, FluoProbe and any derivative thereof, Fluorescein and any derivative thereof, Fura and any derivative thereof, GelGreen and any derivative thereof, GelRed and any derivative thereof, fluorescent proteins and any derivative thereof, m isoform proteins and any derivative thereof such as for example mCherry, hetamethine dye and any derivative thereof, hoeschst stain, iminocoumarin, indian yellow, indo-1 and any derivative thereof, laurdan, lucifer yellow and any derivative thereof, luciferin and any derivative thereof, luciferase and any derivative thereof, mercocyanine and any derivative thereof, nile dyes and any derivative thereof, perylene, phloxine, phyco dye and any derivative thereof, propium iodide, pyranine, rhodamine and any derivative thereof, ribogreen, RoGFP, rubrene, stilbene and any derivative thereof, sulforhodamine and any derivative thereof, SYBR™ and any derivative thereof, synapto-pHluorin, tetraphenyl butadiene, tetrasodium tris, Texas Red, Titan Yellow, TSQ, umbelliferone, violanthrone, yellow fluroescent protein and YOYO-1. Other Suitable fluorescent dyes include, but are not limited to, fluorescein and fluorescein dyes (e.g., fluorescein isothiocyanine or FITC, naphthofluorescein, 4', 5'-dichloro-2',7'-dimethoxyfluorescein, 6-carboxyfluorescein or FAM, etc.), carbocyanine, merocyanine, styryl dyes, oxonol dyes, phycoerythrin, erythrosin, eosin, rhodamine dyes (e.g., carboxytetramethyl-rhodamine or TAMRA, carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), lissamine rhodamine B, rhodamine 6G, rhodamine Green, rhodamine Red, tetramethylrhodamine (TMR), etc.), coumarin and coumarin dyes (*e.g.*, methoxycoumarin, dialkylaminocoumarin, hydroxycoumarin, aminomethylcoumarin (AMCA), etc.), Oregon

Green® Dyes (*e.g.*, Oregon Green® 488, Oregon Green® 500, Oregon Green® 514., etc.), Texas Red, Texas Red-X, SPECTRUM RED, SPECTRUM GREEN, cyanine dyes (e.g., CY-3, Cy-5, CY-3.5, CY-5.5, etc.), ALEXA FLUOR® dyes (*e.g.*, ALEXA FLUOR® 350, ALEXA FLUOR® 488, ALEXA FLUOR® 532, ALEXA FLUOR® 546, ALEXA FLUOR® 568, ALEXA FLUOR® 594, ALEXA FLUOR® 633, ALEXA FLUOR® 660, ALEXA FLUOR® 680, etc.), BODIPY® dyes (*e.g.*, BODIPY® FL, BODIPY® R6G, BODIPY® TMR, BODIPY® TR, BODIPY® 530/550, BODIPY® 558/568, BODIPY® 564/570, BODIPY® 576/589, BODIPY® 581/591, BODIPY® 630/650, BODIPY® 650/665, etc.), IRDyes (*e.g.*, IRD40, IRD 700, IRD 800, etc.), and the like. Additional suitable detectable agents are described in PCT/US14/56177. Non-limiting examples of radioisotopes include alpha emitters, beta emitters, positron emitters, and gamma emitters. In some embodiments, the metal or radioisotope is selected from the group consisting of actinium, americium, bismuth, cadmium, cesium, cobalt, europium, gadolinium, iridium, lead, lutetium, manganese, palladium, polonium, radium, ruthenium, samarium, strontium, technetium, thallium, and yttrium. In some embodiments, the metal is actinium, bismuth, lead, radium, strontium, samarium, or yttrium. In some embodiments, the radioisotope is actinium-225 or lead-212.

A peptide may be conjugated to a radiosensitizer or photosensitizer. Examples of radiosensitizers include but are not limited to: ABT-263, ABT-199, WEHI-539, paclitaxel, carboplatin, cisplatin, oxaliplatin, gemcitabine, etanidazole, misonidazole, tirapazamine, and nucleic acid base derivatives (*e.g.*, halogenated purines or pyrimidines, such as 5-fluorodeoxyuridine). Examples of photosensitizers include but are not limited to: fluorescent molecules or beads that generate heat when illuminated, nanoparticles, porphyrins and porphyrin derivatives (*e.g.*, chlorins, bacteriochlorins, isobacteriochlorins, phthalocyanines, and naphthalocyanines), metalloporphyrins, metallophthalocyanines, angelicins, chalcogenapyrrillium dyes, chlorophylls, coumarins, flavins and related compounds such as alloxazine and riboflavin, fullerenes, pheophorbides, pyropheophorbides, cyanines (*e.g.*, merocyanine 540), pheophytins, sapphyrins, texaphyrins, purpurins, porphycenes, phenothiaziniums, methylene blue derivatives, naphthalimides, nile blue derivatives, quinones, perylenequinones (*e.g.*, hypericins, hypocrellins, and cercosporins), psoralens, quinones, retinoids, rhodamines, thiophenes, verdins, xanthene dyes (*e.g.*, eosins, erythrosins, rose bengals), dimeric and oligomeric forms of porphyrins, and prodrugs such as 5-aminolevulinic acid. Advantageously, this approach allows for highly specific targeting of cells of interest (*e.g.*, immune cells) using both a therapeutic agent (*e.g.*, drug) and

electromagnetic energy (*e.g.*, radiation or light) concurrently. In some embodiments, the peptide is fused with, or covalently or non-covalently linked to the agent, for example, directly or via a linker.

In some embodiments, the binding protein may be chemically modified. For example, a binding protein may be mutated to modify peptide properties such as detectability, stability, biodistribution, pharmacokinetics, half-life, surface charge, hydrophobicity, conjugation sites, pH, function, and the like. N-methylation is one example of methylation that can occur in a binding protein encompassed by the present invention. In some embodiments, a binding protein may be modified by methylation on free amines such as by reductive methylation with formaldehyde and sodium cyanoborohydride.

A chemical modification may comprise a polymer, a polyether, polyethylene glycol, a biopolymer, a zwitterionic polymer, a polyamino acid, a fatty acid, a dendrimer, an Fc region, a simple saturated carbon chain such as palmitate or myristolate, or albumin. The chemical modification of a binding protein with an Fc region may be a fusion Fc-protein. A polyamino acid may include, for example, a poly amino acid sequence with repeated single amino acids (*e.g.*, poly glycine), and a poly amino acid sequence with mixed poly amino acid sequences that may or may not follow a pattern, or any combination of the foregoing.

In some embodiments, the binding proteins encompassed by the present invention may be modified. In some embodiments, the modifications having substantial or significant sequence identity to a parent binding protein to generate a functional variant that maintains one or more biophysical and/or biological activities of the parent binding protein (*e.g.*, maintain pMHC binding specificity). In some embodiments, the mutation is a conservative amino acid substitution.

In some embodiments, binding proteins encompassed by the present invention may comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are well-known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, a-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, l ,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, a-aminocyclopentane carboxylic acid, oc-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic acid, a-(2-amino-2-

norbornane)-carboxylic acid, α,γ-diaminobutyric acid, ,β-diaminopropionic acid, homophenylalanine, and oc-tert-butylglycine.

Binding proteins encompassed by the present invention may be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized (*e.g.*, via a disulfide bridge), or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

In some embodiments, the attachment of a hydrophobic moiety, such as to the N-terminus, the C-terminus, or an internal amino acid, may be used to extend half-life of a peptide encompassed by the present invention. In other embodiments, a binding protein may include post-translational modifications (*e.g.*, methylation and/or amidation), which can affect, for example, serum half-life. In some embodiments, simple carbon chains (*e.g.*, by myristoylation and/or palmitylation) may be conjugated to the binding proteins. In some embodiments, the simple carbon chains may render the binding proteins easily separable from the unconjugated material. For example, methods that may be used to separate the binding proteins from the unconjugated material include, but are not limited to, solvent extraction and reverse phase chromatography. The lipophilic moieties can extend half-life through reversible binding to serum albumin. The conjugated moieties may be lipophilic moieties that extend half-life of the peptides through reversible binding to serum albumin. In some embodiments, the lipophilic moiety may be cholesterol or a cholesterol derivative, including cholestenes, cholestanes, cholestadienes and oxysterols. In some embodiments, the binding proteins may be conjugated to myristic acid (tetradecanoic acid) or a derivative thereof. In other embodiments, a binding protein may be coupled (*e.g.*, conjugated) to a half-life modifying agent. Examples of half-life modifying agents include but are not limited to: a polymer, a polyethylene glycol (PEG), a hydroxyethyl starch, polyvinyl alcohol, a water soluble polymer, a zwitterionic water soluble polymer, a water soluble poly(amino acid), a water soluble polymer of proline, alanine and serine, a water soluble polymer containing glycine, glutamic acid, and serine, an Fc region, a fatty acid, palmitic acid, or a molecule that binds to albumin. In some embodiments, a spacer or linker may be coupled to a binding protein, such as 1, 2, 3, 4, or more amino acid residues that serve as a spacer or linker in order to facilitate conjugation or fusion to another molecule, as well as to facilitate cleavage of the peptide from such conjugated or fused molecules. In some embodiments, binding proteins may be conjugated to other moieties that, for example, can modify or effect changes to the properties of the binding proteins.

A protein such as a peptide may be produced recombinantly or synthetically, such as by solid-phase peptide synthesis or solution-phase peptide synthesis. Protein synthesis may be performed by known synthetic methods, such as using fluorenylmethyloxycarbonyl (Fmoc) chemistry or by butyloxycarbonyl (Boc) chemistry. Protein fragments may be joined together enzymatically or synthetically.

In an aspect encompassed by the present invention, provided herein are methods of producing a protein described herein, comprising the steps of: (i) culturing a transformed host cell which has been transformed by a nucleic acid comprising a sequence encoding a binding protein described herein under conditions suitable to allow expression of said binding protein; and (ii) recovering the expressed binding protein.

Methods useful for isolating and purifying recombinantly produced binding protein, by way of example, may include obtaining supernatants from suitable host cell/vector systems that secrete the binding protein 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 binding proteins described herein include batch cell culture, which is monitored and controlled to maintain appropriate culture conditions. Purification of the binding protein may be performed according to methods described herein and known in the art.

In some embodiments, provided herein is a nucleic acid encoding a MAGEC2 immunogenic peptide described herein or fragment thereof, such as a DNA molecule encoding a MAGEC2 immunogenic peptide. In some embodiments, the composition comprises an expression vector comprising an open reading frame encoding a MAGEC2 immunogenic peptide described herein or fragment thereof. In some embodiments, the nucleic acid includes regulatory elements necessary for expression of the open reading frame. Such elements may include, for example, a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers may be included. These elements may be operably linked to a sequence that encodes the MAGEC2 immunogenic polypeptide or fragment thereof. Representative vectors, promoters, regulatory elements, and the like useful for expressing proteins such as peptide are described further below.

III. MHC-peptide complexes

In certain aspects, provided herein are compositions comprising a MAGEC2 immunogenic peptide described herein and a MHC molecule. In some embodiments, the MAGEC2 immunogenic peptide forms a stable complex with the MHC molecule.

MHC proteins may be conjugated to an agent, such as a detection moiety, readiosensitizer, photosensitizer, and the like, and/or may be chemically modified as described above regarding peptides.

The MHC proteins provided and used in the compositions and methods encompassed by the present disclosure may be any suitable MHC molecules known in the art. Generally, they have the formula $(\alpha\text{-}\beta\text{-}P)_n$, where n is at least 2, for example between 2-10, *e.g.,* 4. α is an α chain of a class I or class II MHC protein. β is a β chain, herein defined as the β chain of a class II MHC protein or β_2 microglobulin for a MHC class I protein. P is a peptide antigen.

In some embodiments, the MHC proteins are MHC class I complexes, such as HLA I complexes.

The MHC proteins may be from any mammalian or avian species, *e.g.,* primate sp., particularly humans; rodents, including mice, rats and hamsters; rabbits; equines, bovines, canines, felines; etc. For instance, the MHC protein may be derived the human HLA proteins or the murine H-2 proteins. HLA proteins include the class II subunits HLA-DPα, HLA-DPβ, HLA-DQα, HLA-DQβ, HLA-DRα and HLA-DRβ, and the class I proteins HLA-A, HLA-B, HLA-C, and β2-microglobulin. H-2 proteins include the class I subunits H-2K, H-2D, H-2L, and the class II subunits I-Aα, I-Aβ, I-Eα and I-Eβ, and β2-microglobulin. Sequences of some representative MHC proteins may be found in Kabat *et al.* Sequences of Proteins of Immunological Interest, NIH Publication No. 91-3242, pp724-815. MHC protein subunits suitable for use in the present invention are a soluble form of the normally membrane-bound protein, which is prepared as known in the art, for instance by deletion of the transmembrane domain and the cytoplasmic domain.

For class I proteins, the soluble form may include the α1, α2 and α3 domain. Soluble class II subunits may include the α1 and α2 domains for the α subunit, and the β1 and β2 domains for the β subunit.

The α and β subunits may be separately produced and allowed to associate *in vitro* to form a stable heteroduplex complex, or both of the subunits may be expressed in a single cell. Methods for producing MHC subunits are known in the art.

In certain embodiments, the MHC-peptide complex comprises a peptide epitope selected from Table 1 and an MHC. In some embodiments, the MHC molecule comprises an

MHC alpha chain that is an HLA-A*B07 serotype, optionally wherein the HLA allele is HLA-B*07:02. In some embodiments, the the MHC-peptide complex comprises a peptide epitope selected from Table 1 and an MHC whose alpha chain has an HLA-B*07 serotype, such as that encoded by an HLA-B*0702.

To prepare the MHC-peptide complex, the subunits may be combined with an antigenic peptide and allowed to fold *in vitro* to form a stable heterodimer complex with intrachain disulfide bonded domains. The peptide may be included in the initial folding reaction, or may be added to the empty heterodimer in a later step. In the compositions and methods encompassed by the present invention, this is a MAGEC2 immunogenic peptide or fragment thereof. Conditions that permit folding and association of the subunits and peptide are known in the art. As one example, roughly equimolar amounts of solubilized α and β subunits may be mixed in a solution of urea. Refolding is initiated by dilution or dialysis into a buffered solution without urea. Peptides may be loaded into empty class II heterodimers at about pH 5 to 5.5 for about 1 to 3 days, followed by neutralization, concentration and buffer exchange. However, the specific folding conditions are not critical for the practice of the invention.

The monomeric complex (α-β-P) (herein monomer) may be multimerized, for example, for a MHC tetramer. The resulting multimer is stable over long periods of time. Preferably, the multimer may be formed by binding the monomers to a multivalent entity through specific attachment sites on the α or β subunit, as known in the art (*e.g.,* as described in U.S. Patent No. 5,635,363). The MHC proteins, in either their monomeric or multimeric forms, may also be conjugated to beads or any other support.

The multimeric complex may be labeled, so as to be directly detectable when used in immunostaining or other methods known in the art, or may be used in conjunction with secondary labeled immunoreagents which specifically and/or selectively bind the complex (*e.g.,* bind to a MHC protein subunit) as known in the art. For example, the detectable label may be a fluorophore, such as fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin (PE), allophycocyanin (APC), Brilliant Violet™ 421, Brilliant UV™ 395, Brilliant Violet™ 480, Brilliant Violet™ 421 (BV421), Brilliant Blue™ 515, APC-R700, or APC-Fire750. In some embodiments, the multimeric complex is labeled by a moiety that is capable of specifically and/or selectively binding another moiety. For instance, the label may be biotin, streptavidin, an oligonucleotide, or a ligand. Other labels of interest may include fluorochromes, dyes, enzymes, chemiluminescers, particles, radioisotopes, or other directly or indirectly detectable agent.

In some embodiments, a cell presenting an immunogenic peptides in context of an MHC molecule on the cell surface is generated by transfecting or transducing the cell with a vector (*e.g.,* a viral vector) that comprising nucleic acid that encodes a recombinant or heterologous antigen into a cell. In some embodiments, the vector is introduced into the cell under conditions in which one or more peptide antigens, including, in some cases, one or more peptide antigens of the expressed heterologous protein, are expressed by the cell, processed and presented on the surface of the cell in the context of a major histocompatibility complex (MHC) molecule.

Generally, the cell to which the vector is contacted is a cell that expresses MHC, *i.e.,* MHC-expressing cells. The cell may be one that normally expresses an MHC on the cell surface, that is induced to express and/or upregulate expression of MHC on the cell surface or that is engineered to express an MHC molecule on the cell surface. In some embodiments, the MHC contains a polymorphic peptide binding site or binding groove that can, in some cases, complex with peptide antigens of polypeptides, including peptide antigens processed by the cell machinery. In some cases, MHC molecules may be displayed or expressed on the cell surface, including as a complex with peptide, *i.e.,* MHC-peptide complex, for presentation of an antigen in a conformation recognizable by TCRs on T cells, or other peptide binding molecules.

In some embodiments, the cell is a nucleated cell. In some embodiments, the cell is an antigen-presenting cell. In some embodiments, the cell is a macrophage, dendritic cell, B cell, endothelial cell or fibroblast. In some embodiments, the cell is an endothelial cell, such as an endothelial cell line or primary endothelial cell. In some embodiments, the cell is a fibroblast, such as a fibroblast cell line or a primary fibroblast cell.

In some embodiments, the cell is an artificial antigen presenting cell (aAPC). Typically, aAPCs include features of natural APCs, including expression of an MHC molecule, stimulatory and costimulatory molecule(s), Fc receptor, adhesion molecule(s) and/or the ability to produce or secrete cytokines (*e.g.,* IL-2). Normally, an aAPC is a cell line that lacks expression of one or more of the above, and is generated by introduction (*e.g.,* by transfection or transduction) of one or more of the missing elements from among an MHC molecule, a low affinity Fc receptor (CD32), a high affinity Fc receptor (CD64), one or more of a co-stimulatory signal (*e.g.,* CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, ICOS-L, ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, MICB, HVEM, lymphotoxin beta receptor, ILT3, ILT4, 3/TR6 or a ligand of B7-H3; or an antibody that specifically binds to CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, LFA-1, CD2,

CD7, LIGHT, NKG2C, B7-H3, Toll ligand receptor or a ligand of CD83), a cell adhesion molecule (*e.g.,* ICAM-1 or LFA-3) and/or a cytokine (*e.g.,* IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-21, interferon-alpha (IFN.alpha.), interferon-beta (IFN.beta.), interferon-gamma (IFN.gamma.), tumor necrosis factor-alpha (TNF.alpha.), tumor necrosis factor-beta (TNF.beta.), granulocyte macrophage colony stimulating factor (GM-CSF), and granulocyte colony stimulating factor (GCSF)). In some cases, an aAPC does not normally express an MHC molecule, but may be engineered to express an MHC molecule or, in some cases, is or may be induced to express an MHC molecule, such as by stimulation with cytokines. In some cases, aAPCs also may be loaded with a stimulatory ligand, which may include, for example, an anti-CD3 antibody, an anti-CD28 antibody or an anti-CD2 antibody. An exemplary cell line that may be used as a backbone for generating an aAPC is a K562 cell line or a fibroblast cell line. Various aAPCs are known in the art, see *e.g.,* U.S. Pat. No. 8,722,400, published application No. US2014/0212446; Butler and Hirano (2014) *Immunol Rev.* 257:10.1111/imr.12129; Suhoshki *et al.* (2007) *Mol. Ther.* 15:981-988).

It is well within the level of a skilled artisan to determine or identify the particular MHC or allele expressed by a cell. In some embodiments, prior to contacting cells with a vector, expression of a particular MHC molecule may be assessed or confirmed, such as by using an antibody specific for the particular MHC molecule. Antibodies to MHC molecules are known in the art, such as any described below.

In some embodiments, the cells may be chosen to express an MHC allele of a desired MHC restriction. In some embodiments, the MHC typing of cells, such as cell lines, are well-known in the art. In some embodiments, the MHC typing of cells, such as primary cells obtained from a subject, may be determined using procedures well-known in the art, such as by performing tissue typing using molecular haplotype assays (BioTest ABC SSPtray, BioTest Diagnostics Corp., Denville, N.J.; SeCore Kits, Life Technologies, Grand Island, N.Y.). In some cases, it is well within the level of a skilled artisan to perform standard typing of cells to determine the HLA genotype, such as by using sequence-based typing (SBT) (Adams *et al.* (2004) J. Transl. Med., 2:30; Smith (2012) Methods Mol Biol., 882:67-86). In some cases, the HLA typing of cells, such as fibroblast cells, are known. For example, the human fetal lung fibroblast cell line MRC-5 is HLA-A*02:01, A29, B13, B44 Cw7 (C*0702); the human foreskin fibroblast cell line Hs68 is HLA-A1, A29, B8, B44, Cw7, Cw16; and the WI-38 cell line is A*68:01, B*08:01 (Solache *et al.* (1999) J Immunol, 163:5512-5518; Ameres *et al.* (2013) PloS Pathog. 9:e1003383). The human transfectant

fibroblast cell line M1DR1/Ii/DM express HLA-DR and HLA-DM (Karakikes *et al.* (2012) FASEB J., 26:4886-96).

In some embodiments, the cells to which the vector is contacted or introduced are cells that are engineered or transfected to express an MHC molecule. In some embodiments, cell lines may be prepared by genetically modifying a parental cells line. In some embodiments, the cells are normally deficient in the particular MHC molecule and are engineered to express such particular MHC molecule. In some embodiments, the cells are genetically engineered using recombinant DNA techniques.

In some embodiments, the stable MHC-peptide complexes described herein are used to detect T cells that bind a stable MHC-peptide complex. In some embodiments, the stable MHC-peptide complexes described herein are used to monitor T cell response in a subject, for example, by detecting the amount and/or percentage of T cells (*e.g.,* CD8+ T cells) that specifically and/or selectively bind to the MHC-peptide complexes that are fluorescently labeled. Methods of generating, labeling, and using MHC-peptide complexes (*e.g.,* MHC-peptide tetramers) for detecting MHC-peptide complex-specific T cells are well-known in the art. Additional description can be found in, for example, U.S. Pat. No. 7,776,562; U.S. Pat. No. 8,268,964; and U.S. Pat. Publ. 2019/0085048.

IV. Immunogenic compositions

In some aspects, provided herein are pharmaceutical compositions (*e.g.,* a vaccine composition) comprising a MAGEC2 immunogenic peptide and/or a nucleic acid encoding a MAGEC2 immunogenic peptide and an adjuvant. In some aspects, provided herein are pharmaceutical compositions (*e.g.,* a vaccine composition) comprising a stable MHC-peptide complex comprising a MAGEC2 immunogenic peptide in the context of a MHC molecule and an adjuvant. In some embodiments, the composition includes a combination of multiple (*e.g.,* two or more) MAGEC2 immunogenic peptides or nucleic acids and an adjuvant. In some embodiments, the composition includes a combination of multiple (*e.g.,* two or more) stable MHC-peptide complexes comprising a MAGEC2 immunogenic peptide in the context of a MHC molecule and an adjuvant. In some embodiments, the compositions described above further comprises a pharmaceutically acceptable carrier.

The pharmaceutical compositions disclosed herein may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.,* those targeted for buccal, sublingual, and systemic absorption, boluses, powders,

granules, pastes for application to the tongue; or (2) parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation.

Methods of preparing these formulations or compositions include the step of bringing into association a MAGEC2 immunogenic peptide and/or nucleic acid described herein with the adjuvant, carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association an agent described herein with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Pharmaceutical compositions suitable for parenteral administration comprise MAGEC2 immunogenic peptides and/or nucleic acids described herein in combination with a adjuvant, as well as one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain sugars, alcohols, antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Regardless of the route of administration selected, the agents provided herein, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions disclosed herein, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

In some embodiments, the pharmaceutical composition described, when administered to a subject, can elicit an immune response against a cell that is infected by MAGEC2. Such pharmaceutical compositions may be useful as vaccine compositions for prophylactic and/or therapeutic treatment of disorders characterized by MAGEC2 expression.

In some embodiments, the pharmaceutical composition further comprises a physiologically acceptable adjuvant. In some embodiments, the adjuvant employed provides for increased immunogenicity of the pharmaceutical composition. Such a further immune

response stimulating compound or adjuvant may be (i) admixed to the pharmaceutical composition according to the invention after reconstitution of the peptides and optional emulsification with an oil-based adjuvant as defined above, (ii) may be part of the reconstitution composition of the invention defined above, (iii) may be physically linked to the peptide(s) to be reconstituted or (iv) may be administered separately to the subject, mammal or human, to be treated. The adjuvant may be one that provides for slow release of antigen (*e.g.,* the adjuvant may be a liposome), or it may be an adjuvant that is immunogenic in its own right thereby functioning synergistically with antigens (*i.e.,* antigens present in the MAGEC2 immunogenic peptide). For example, the adjuvant may be a known adjuvant or other substance that promotes antigen uptake, recruits immune system cells to the site of administration, or facilitates the immune activation of responding lymphoid cells. Adjuvants include, but are not limited to, immunomodulatory molecules (*e.g.,* cytokines), oil and water emulsions, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, Bacto-Adjuvant, synthetic polymers such as poly amino acids and co-polymers of amino acids, saponin, paraffin oil, and muramyl dipeptide. In some embodiments, the adjuvant is Adjuvant 65, α-GalCer, aluminum phosphate, aluminum hydroxide, calcium phosphate, β-Glucan Peptide, CpG DNA, GM-CSF, GPI-0100, IFA, IFN-γ, IL-17, lipid A, lipopolysaccharide, Lipovant, Montanide, N-acetyl-muramyl-L-alanyl-D-isoglutamine, Pam3CSK4, quil A, trehalose dimycolate or zymosan.

In some embodiments, the adjuvant is an immunomodulatory molecule. For example, the immunomodulatory molecule may be a recombinant protein cytokine, chemokine, or immunostimulatory agent or nucleic acid encoding cytokines, chemokines, or immunostimulatory agents designed to enhance the immunologic response.

Examples of immunomodulatory cytokines include interferons (*e.g.,* IFNα, IFNβ and IFNγ), interleukins (*e.g.,* IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-17 and IL-20), tumor necrosis factors (*e.g.,* TNFα and TNFβ), erythropoietin (EPO), FLT-3 ligand, gIp10, TCA-3, MCP-1, MIF, MIP-1.alpha., MIP-1β, Rantes, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF), as well as functional fragments of any of the foregoing.

In some embodiments, an immunomodulatory chemokine that binds to a chemokine receptor, *i.e.,* a CXC, CC, C, or CX3C chemokine receptor, also may be included in the compositions provided here. Examples of chemokines include, but are not limited to, Mip1α, Mip-1β, Mip-3α (Larc), Mip-3β, Rantes, Hcc-1, Mpif-1, Mpif-2, Mcp-1, Mcp-2, Mcp-3,

Mcp-4, Mcp-5, Eotaxin, Tarc, Elc, I309, IL-8, Gcp-2 Gro-α, Gro-β, Gro-γ, Nap-2, Ena-78, Gcp-2, Ip-10, Mig, I-Tac, Sdf-1, and Bca-1 (Blc), as well as functional fragments of any of the foregoing.

In some embodiments, the composition comprises a nucleic acid encoding an MAGEC2 immunogenic polypeptide described herein, such as a DNA molecule encoding a MAGEC2 immunogenic peptide. In some embodiments the composition comprises an expression vector comprising an open reading frame encoding a MAGEC2 immunogenic peptide.

When taken up by a cell (*e.g.*, host cell, an antigen-presenting cell (APC) such as a dendritic cell, macrophage, etc.), a DNA molecule may be present in the cell as an extrachromosomal molecule and/or may integrate into the chromosome. DNA may be introduced into cells in the form of a plasmid which may remain as separate genetic material. Alternatively, linear DNAs that may integrate into the chromosome may be introduced into the cell. Optionally, when introducing DNA into a cell, reagents which promote DNA integration into chromosomes may be added.

V. Binding Proteins

In some aspects, a binding moiety that binds a peptide described herein and/or a stable MHC-peptide complex described herein, are provided. For example, binding proteins like T cell receptors (TCRs), antibodies, and the like that specifically and/or selectively bind to the peptide and/or the stable MHC-peptide complex, such as with a K_d less than or equal to about 10^{-4} M (*e.g.*, about 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, about 10^{-8}, about 10^{-9}, about 10^{10}, about 10^{-11}, about 10^{-12}, about 10^{-13}, about 10^{-14}, etc.), are provided.

In an aspect encompassed by the present invention, provided herein are binding proteins that bind (*e.g.*, specifically and/or selectively) to a peptide-MHC (pMHC) complex comprising a MAGEC2 immunogenic peptide in the context of an MHC molecule (*e.g.*, an MHC class I molecule). In some embodiments, the binding protein is capable of binding (*e.g.*, specifically and/or selectively) to a MAGEC2 peptide-MHC (pMHC) complex with a K_d less than or equal to about $5x10^{-4}$ M, less than or equal to about $1x10^{-4}$ M, less than or equal to about $5x10^{-5}$ M, less than or equal to about $1x10^{-5}$ M, less than or equal to about $5x10^{-6}$ M, less than or equal to about $1x10^{-6}$ M, less than or equal to about $5x10^{-7}$ M, less than or equal to about $1x10^{-7}$ M, less than or equal to about $5x10^{-8}$ M, less than or equal to about $1x10^{-8}$ M, less than or equal to about $5x10^{-9}$ M, less than or equal to about $1x10^{-9}$ M, less than or equal to about $5x10^{-10}$ M, less than or equal to about $1x10^{-10}$ M, less than or equal to about

$5x10^{-11}$ M, less than or equal to about $1x10^{-11}$ M, less than or equal to about $5x10^{-12}$ M, less than or equal to about $1x10^{-12}$ M, or any range in between, inclusive, such as between about 1-50 micromolar, 1-100 micromolar, 0.1-500 micromolar, and the like. In some embodiments, the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702. In some embodiments, the binding proteins provided herein are genetically engineered, isolated, and/or purified.

In some embodiments, the binding proteins have a higher binding affinity to the MAGEC2 peptide-MHC (pMHC) than does a known T-cell receptor (*e.g.*, a TCR from van Kunert *et al.* (2016) *J. Immunol.* 197:2541-2552 or others described herein). For example, the binding proteins may have at least 1.2 fold, 1.5 fold, 1.8 fold, 2.0 fold, 2.2 fold, 2.5 fold, 2.8 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 5.5 fold, 6 fold, 6.5 fold, 7 fold, 7.5 fold, 8 fold, 8.5 fold, 9 fold, 9.5 fold, 10 fold, 11 fold, 12 fold, 13 fold, 14 fold, 15 fold, 16 fold, 17 fold, 18 fold, 19 fold, 20 fold, 25 fold, 30 fold, 35 fold, 40 fold, 45 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 1000 fold, 5000 fold, 10000 fold, 50000 fold, 100000 fold, 500000 fold, 1000000 fold, or more, or any range in between, inclusive, such as 1.2 fold to 2 fold, higher binding affinity to the MAGEC2 peptide-MHC (pMHC) than does a known T-cell receptor.

In some embodiments, the binding protein induces higher T cell expansion, cytokine release, and/or cytotoxic killing than does a known T-cell receptor when contacted with target cells with expression of MAGEC2 at a certain level or below (*e.g.*, see the Examples section for representative cell lines expressing MAGEC2 at varying levels). For example, in some embodiments of any aspect described herein, MAGEC2 level can be expressed in terms of transcripts per million and may be, for example, less than or equal to about 1,000 transcript per million transcripts (TPM), 950 TPM, 900 TPM, 850 TPM, 800 TPM, 750 TPM, 700 TPM, 650 TPM, 600 TPM, 550 TPM, 500 TPM, 450 TPM, 400 TPM, 350 TPM, 300 TPM, 250 TPM, 200 TPM, 150 TPM, 100 TPM, 95 TPM, 90 TPM, 85 TPM, 80 TPM, 75 TPM, 70 TPM, 65 TPM, 60 TPM, 55 TPM, 50 TPM, 45 TPM, 40 TPM, 35 TPM, 34 TPM, 33 TPM, 32 TPM, 31 TPM, 30 TPM, 29 TPM, 28 TPM, 27 TPM, 26 TPM, 25 TPM, 24 TPM, 23 TPM, 22 TPM, 21 TPM, 20 TPM, 19 TPM, 18 TPM, 17 TPM, 16 TPM, 15 TPM, 14 TPM, 13 TPM, 12 TPM, 11 TPM, 10 TPM, 9 TPM, 8 TPM, 7 TPM, 6 TPM, 5 TPM, 4 TPM, 3 TPM, 2 TPM, and 1 TPM, or any range in between, inclusive, such as less than or equal to about 1,000 TPM to less than or equal to about 73 TPM). In some embodiments, the low MAGEC2 expression level is termed "heterozygous expression" meaning between about 1 TPM and about 73 TPM, or any range in between, inclusive, such as 73 TPM or 1-73 TPM.

A higher expression is 74 TPM and higher. As described further herein, TPM is measured according to well-known techniques, such as RNA-Seq, and gene expression TPM data are well-known in the art for a variety of cell lines, tissue types, and the like (see, for example, the Broad Institute Cancer Cell Line Encyclopedia (CCLE) on the World Wide Web at portals.broadinstitute.org). In some embodiment, the binding protein induces at least 1.2 fold, 1.5 fold, 1.8 fold, 2.0 fold, 2.2 fold, 2.5 fold, 2.8 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 5.5 fold, 6 fold, 6.5 fold, 7 fold, 7.5 fold, 8 fold, 8.5 fold, 9 fold, 9.5 fold, 10 fold, 11 fold, 12 fold, 13 fold, 14 fold, 15 fold, 16 fold, 17 fold, 18 fold, 19 fold, 20 fold, 25 fold, 30 fold, 35 fold, 40 fold, 45 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 1000 fold, or more, or any range in between, inclusive, such as 1.2 fold to 2 fold, increase in T cell expansion (*e.g.*, proflieration), cytokine release, and/or cytotoxic killing than does a known T-cell receptor (*e.g.*, a comparator TCR described herein) when contacted with target cells expressing MAGEC2 peptide epitope, such as with heterozygous expression of MAGEC2 peptide epitope.

In some embodiments, the expression of MAGEC2 is detected using RNA-sequencing (RNA-seq). RNA-seq generally comprises the following steps: obtaining a sample containing genetic material, isolating total RNA from the sample obtained, preparing an amplified cDNA library from the total RNA, sequencing the amplified cDNA library, and analyzing and profiling the amplified cDNA to assess the expression level of different transcripts. The sample can be a population of cells, a tissue sample, a bioposy sample, a cell culture, or a single cell. Total RNA can be isolated from the biological sample using any method known in the art. In certain embodiments, total RNA is extracted from plasma. Plasma RNA extraction is described in Enders et al., "The Concentration of Circulating Corticotropin-Releasing Homer mRNA in Material Plasma Is Inclined in Preclampsia," Clinr. As described therein, the plasma collected after the centrifugation step is mixed with Trizol LS reagent (Invitrogen) and chloroform. The mixture is centrifuged and the aqueous layer is transferred to a new tube. Ethanol is added to this aqueous layer. The mixture is then placed in an RNeasy mini column (Qiagen) and processed according to the manufacturer's recommendations.

In some embodiments, RNA-seq described herein includes the step of preparing amplified cDNA from total RNA. For example, cDNA is prepared and the isolated RNA sample is randomly amplified without dilution, or the mixture of genetic material in the isolated RNA is dispersed into individual reaction samples. In certain embodiments, amplification is initiated randomly at the 3 'end and throughout the entire

transcriptome in the sample to amplify both mRNA and non-polyadenylated transcripts. In this way, double-stranded cDNA amplification products are optimized for the generation of sequencing libraries for next generation sequencing platforms. A kit suitable for amplification of cDNA by the method encompassed by the present invention includes, for example, Ovation® RNA-Seq System.

In some embodiments, RNA-seq described herein includes the step of sequencing the amplified cDNA. Any known sequencing method can be used to sequence the amplified cDNA mixture including the single molecule sequencing method. In certain embodiments, the amplified cDNA is sequenced by whole transcriptome shotgun sequencing. Whole transcriptome shotgun sequencing can be performed using various next generation sequencing platforms such as Illumina® Genome Analyzer platform, ABI SOLiD™ Sequencing platform, or Life Science's 454 Sequencing platform.

In some embodiments, RNA-seq described herein further comprises performing digital counting and analysis on the cDNA. The number of amplified sequences for each transcript in the amplified sample can be quantified by sequence reading (one reading per amplified strand). In some embodiments, transcript per million (TPM) is used to quantify the expression level of a particular transcript. TPM may be calculated as shown in Wagner *et al.* (2012) *Theory in Biosciences* 131:281-285, the content of which is incorporated by reference herein in its entirety.

In certain embodiments, the binding proteins recognize a MAGEC2 immunogenic peptide in a complex with MHC molecules, such as particular HLA molecules having particular HLA alpha chain alleles. For example, binding proteins listed in Table 2 were identified as binders of MAGEC2 immunogenic peptides in association with an MHC whose alpha chain had an HLA-B*07 serotype, such as that encoded by an HLA-B*0702, as described further in the Examples section. In some embodiments, the binding proteins recognize a complex of MAGEC2 immunogenic peptide and an MHC molecule, wherein the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702. In some embodiments, the MAGEC2 immunogenic peptides are derived from a human MAGEC2 protein and/or a MAGEC2 protein shown in Table 3. In some embodiments, one or more MAGEC2 immunogenic peptides are administered alone or in combination with an adjuvant.

In some embodiments, the binding proteins do not bind to a peptide-MHC (pMHC) complex, optionally wherein the peptide is derived from an "off-target" described herein.

In some embodiments, the binding protein does not bind to an "off-target" described herein complexed with an MHC peptide-MHC (pMHC) complex.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of): a) a TCR alpha chain sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR alpha chain sequence selected from the group consisting of the TCR alpha sequences listed in Table 2; and/or b) a TCR beta chain sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR beta chain sequence selected from the group consisting of the TCR beta chain sequences listed in Table 2.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of): a) a TCR alpha chain sequence selected from the group consisting of the TCR alpha chain sequences listed in Table 2; and/or b) a TCR beta chain sequence selected from the group consisting of the TCR beta chain sequences listed in Table 2.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of): a) a TCR alpha chain variable (V_α) domain sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR alpha chain variable (V_α) domain sequence selected from the group consisting of the TCR V_α domain sequences listed in Table 2; and/or b) a TCR beta chain variable (V_β) domain sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR beta chain variable (V_β) domain sequence selected from the group consisting of the TCR V_β domain sequences listed in Table 2.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of): a) a TCR alpha chain variable (V_α) domain sequence selected from the group consisting of the TCR V_α domain sequences listed in Table 2; and/or b) a TCR beta chain variable (V_β) domain sequence selected from the group consisting of the TCR V_β domain sequences listed in Table 2.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of at least one (*e.g.*, one, two or three, such as CDR3 alone or in combination with a CDR1 and CDR2)) TCR alpha chain complementarity determining

region (CDR) sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR alpha chain CDR sequence selected from the group consisting of the TCR alpha chain CDR sequences listed in Table 2. CDR3 is believed to be the main CDR responsible for recognizing processed antigen and CDR1 and CDR2 mainly interact with the MHC, so, in some embodiments, binding protein comprising a CDR3 alone from a TCR alpha chain and/or a CDR3 alone from a TCR beta chain listed in Table 2, each CDR3 having a sequence homology as recited in this paragraph, are provided.

In some embodiments, the binding proteins provided herein may also include (*e.g.*, comprise, consist essentially of, or consist of at least one (*e.g.*, one, two or three, such as CDR3 alone or in combination with a CDR1 and CDR2)) TCR beta chain complementarity determining region (CDR) sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR beta chain CDR sequence selected from the group consisting of the TCR beta chain CDR sequences listed in Table 2. As described above, CDR3 is believed to be the main CDR responsible for recognizing processed antigen and CDR1 and CDR2 mainly interact with the MHC, so, in some embodiments, binding protein comprising a CDR3 alone from a TCR beta chain and/or a CDR3 alone from a TCR alpha chain listed in Table 2, each CDR3 having a sequence homology as recited in this paragraph, are provided.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of at least one (*e.g.*, one, two or three)) TCR alpha chain complementarity determining region (CDR) listed in Table 2.

In some embodiments, the binding proteins provided herein may also include (*e.g.*, comprise, consist essentially of, or consist of at least one (*e.g.*, one, two or three)) TCR beta chain complementarity determining region (CDR) listed in Table 2.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of) a TCR alpha chain constant region (C_α) sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR Cα sequence listed in Table 2.

In some embodiments, the binding proteins provided herein may also include (*e.g.*, comprise, consist essentially of, or consist of) a TCR beta chain constant region (C_β) sequence with at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR Cβ sequence listed in Table 2.

In some embodiments, the binding proteins provided herein include (*e.g.*, comprise, consist essentially of, or consist of) a TCR alpha chain constant region (Cα) sequence selected from the group consisting of the TCR Cα sequences listed in Table 2.

In some embodiments, the binding proteins provided herein may also include (*e.g.*, comprise, consist essentially of, or consist of) a TCR beta chain constant region (Cβ) sequence selected from the group consisting of the TCR Cβ sequences listed in Table 2.

Table 2: TCR sequences recognizing a MAGEC2 antigen presented by HLA serotype HLA-B*07

MAGEC2_184-6 MGTM codon optimized sequence (also known as "184-6", "6", "TCR 6", TCR expressed by "TSC-201-B07", and TCR expressed by "TSC-201-B0702")

Alpha chain:
TRAV22/TRAJ44/TRAC

Alpha chain DNA sequence
atgaagagaatcctgggcgccctgctgggactgctgtctgcccaagtctgttgtgtgcggggcatccaggtggaacagagccctcca
gatctgatcctgcaagagggcgccaacagcaccctgagatgcaacttcagc**gactccgtgaacaac**ctgcagtggttccaccagaa
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gacattttgggactggaacaagacttcaggtcacgctcgacATCCAGAACCCCGACCCCGCCGTGTACCA
GCTGAGGGACTCCAAGTCCAGCGACAAGAGCGTGTGTCTGTTTACGGACTTCGAC
AGCCAGACCAACGTGAGTCAAAGCAAGGACAGCGACGTCTACATAACGGATAAG
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TCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCATCATCCCCG
AGGACACCTTCTTCCCCAGCAGCGACGTGCCCTGCGACGTGAAACTGGTGGAGA
AGTCCTTCGAGACAGACACCAATCTGAACTTTCAGAACCTGCTGGTGATCGTGCT
GCGGATTCTGCTGCTGAAAGTGGCCGGCTTCAATCTGCTGATGACCCTGCGGCTG
TGGAGCAGC

Alpha chain Protein sequence
MKRILGALLGLLSAQVCCVRGIQVEQSPPDLILQEGANSTLRCNFS**DSVNN**LQWFHQ
NPWGQLINLFY**IPSGT**KQNGRLSATTVATERYSLLYISSSQTTDSGVYF**CAVTGTAS**
KLTFGTGTRLQVTLDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVY
ITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSSDVPCDVKLV
EKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

Beta chain:
TRBV7-9/TRBJ2-7/TRBC1

Beta chain DNA sequence
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atttttgagcaatattttgggccgggcaccaggctcacggtcacagAAGATCTGAACAAGGTGTTCCCTCC
AGAGGTGGCCGTGTTCGAGCCTTCTAAGGCCGAGATCGCCCACACACAAAAAGC
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TTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAGAGCCAAGCCCGTGAC
ACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCATCACCAGCGC
CTCCTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGAAATCCTGCTGGGC
AAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGCCATGGTCA
AGCGGAAGGACTTT

Beta chain Protein sequence
MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS**
SKGIFEQYFGPGTRLTVTEDLNKVFPPEVAVFEPSKAEIAHTQKATLVCLATGFFPDH
VELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC
QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGITSASYHQGVLSATILYEILL
GKATLYAVLVSALVLMAMVKRKDF

Complete Beta and Alpha ORF DNA Sequence
atgggaacctctctgctgtgctggatggccctgtgtctgctgggagccgatcatgccgatacgggagtgtcccaggatcctcggcaca
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GCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCT
TCAACAACAGCATCATCCCCGAGGACACCTTCTTCCCCAGCAGCGACGTGCCCTG
CGACGTGAAACTGGTGGAGAAGTCCTTCGAGACAGACACCAATCTGAACTTTCA

GAACCTGCTGGTGATCGTGCTGCGGATTCTGCTGCTGAAAGTGGCCGGCTTCAAT
CTGCTGATGACCCTGCGGCTGTGGAGCAGC

Complete Beta and Alpha ORF Protein Sequence
MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS**
SKGIFEQYFGPGTRLTVTEDLNKVFPPEVAVFEPSKAEIAHTQKATLVCLATGFFPDH
VELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC
QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGITSASYHQGVLSATILYEILL
GKATLYAVLVSALVLMAMVKRKDFGSGRAKRSGSGATNFSLLKQAGDVEENPGPM
KRILGALLGLLSAQVCCVRGIQVEQSPPDLILQEGANSTLRCNFS**DSVNN**LQWFHQNP
WGQLINLFY**IPSGT**KQNGRLSATTVATERYSLLYISSSQTTDSGVYF**CAVTGTASKL**
TFGTGTRLQVTLDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYIT
DKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSSDVPCDVKLVEK
SFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

MAGEC2_184-6 wild type sequence

Alpha chain DNA sequence
TRAV22/TRAJ44/TRAC

atgaagaggatattgggagctctgctggggctcttgagtgcccaggtttgctgtgtgagaggaatacaagtggagcagagtcctccag
acctgattctccaggagggagccaattccacgctgcggtgcaatttttct**gactctgtgaacaat**ttgcagtggtttcatcaaaaccctg
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CCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTT
TGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATC
CTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCA
GC

Alpha chain Protein sequence
MKRILGALLGLLSAQVCCVRGIQVEQSPPDLILQEGANSTLRCNFS**DSVNN**LQWFHQ
NPWGQLINLFY**IPSGT**KQNGRLSATTVATERYSLLYISSSQTTDSGVYF**CAVTGTAS**
KLTFGTGTRLQVTLDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVY
ITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVE
KSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Beta chain DNA sequence
TRBV7-9/TRBJ2-7/TRBC1

atgggcaccagcctcctctgctggatggccctgtgtctcctgggggcagatcacgcagatactggagtctcccagaaccccagacac
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attttgagcagtacttcgggccgggcaccaggctcacggtcacagAGGACCTGAACAAGGTGTTCCCACC
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TCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCA
CCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGT
GTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGG
AAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCA
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Beta chain Protein sequence

MGTSLLCWMALCLLGADHADTGVSQNPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS**
SKGIFEQYFGPGTRLTVTEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDH
VELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRC
QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILL
GKATLYAVLVSALVLMAMVKRKDF

MAGEC2_184-422 MGTM codon optimized sequence

Alpha chain:
TRAV13-1/TRAJ56/TRAC

Alpha chain DNA sequence
atgaccagcatccgggccgtgttcatcttcctctggctccaactggacctcgtgaacggcgagaacgtggaacagcatcctccacac
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ctaacagtaagctgacattcggaaaaggaataactctgagtgttagaccagacATCCAGAACCCCGACCCCGCC
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TGGTGGAGAAGTCCTTCGAGACAGACACCAATCTGAACTTTCAGAACCTGCTGGT
GATCGTGCTGCGGATTCTGCTGCTGAAAGTGGCCGGCTTCAATCTGCTGATGACC
CTGCGGCTGTGGAGCAGC

Alpha chain Protein sequence
MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYS**DSASNY**FPWYKQ
ELGKGPQLIID**IRSNVGE**KKDQRIAVTLNKTAKHFSLHITETQPEDSAVYF**CAASIYG**
ANSKLTFGKGITLSVRPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSSDVPCDVK
LVEKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

Beta chain:
TRBV15/TRBJ2-1/TRBC1

Beta chain DNA sequence

atgggccctggactgctgcattggatggctctgtgcctgctcggaacaggacatggcgacgctatggtcattcagaaccccagatacc
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TGGCCATGGTCAAGCGGAAGGACTTT

Beta chain Protein sequence

MGPGLLHWMALCLLGTGHGDAMVIQNPRYQVTQFGKPVTLSCSQT**LNHNV**MYWY
QQKSSQAPKLLFH**YYDKDF**NNEADTPDNFQSRRPNTSFCFLDIRSPGLGDAAMYL**CA**
TSGGGQGLLSSYNEQFFGPGTRLTVLEDLNKVFPPEVAVFEPSKAEIAHTQKATLVC
LATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFW
QNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGITSASYHQGVL
SATILYEILLGKATLYAVLVSALVLMAMVKRKDF

Complete Beta and Alpha ORF DNA Sequence

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AGACACCAATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCGGATTCTGCTG
CTGAAAGTGGCCGGCTTCAATCTGCTGATGACCCTGCGGCTGTGGAGCAGC

Complete Beta and Alpha ORF Protein Sequence
MGPGLLHWMALCLLGTGHGDAMVIQNPRYQVTQFGKPVTLSCSQT**LNHNV**MYWY
QQKSSQAPKLLFH**YYDKDF**NNEADTPDNFQSRRPNTSFCFLDIRSPGLGDAAMYL**CA**
TSGGGQGLLSSYNEQFFGPGTRLTVLEDLNKVFPPEVAVFEPSKAEIAHTQKATLVC
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YFPWYKQELGKGPQLIID**IRSNVGE**KKDQRIAVTLNKTAKHFSLHITETQPEDSAVYF
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VSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSS
DVPCDVKLVEKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

MAGEC2_184-422 wild type sequence

Alpha chain DNA sequence
TRAV13-1/TRAJ56/TRAC

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TGGTCCAGC

Alpha chain Protein sequence
MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYS**DSASNY**FPWYKQ
ELGKRPQLIID**IRSNVGE**KKDQRIAVTLNKTAKHFSLHITETQPEDSAVYF**CAASIYG**
ANSKLTFGKGITLSVRPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSD
VYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL
VEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Beta chain DNA sequence
TRBV15/TRBJ2-1/TRBC1

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CCATGGTCAAGAGAAAGGATTTC

Beta chain Protein sequence

MGPGLLHWMALCLLGTGHGDAMVIQNPRYQVTQFGKPVTLSCSQT**LNHNV**MYWY
QQKSSQAPKLLFH**YYDKDF**NNEADTPDNFQSRRPNTSFCFLDIRSPGLGDAAMYL**CA**
TSGGGQGLLSSYNEQFFGPGTRLTVLEDLNKVFPPEVAVFEPSEAEISHTQKATLVC
LATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFW
QNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGV
LSATILYEILLGKATLYAVLVSALVLMAMVKRKDF

MAGEC2_184-563 MGTM codon optimized sequence

Alpha chain:
TRAV3/TRAJ8/TRAC

Alpha chain DNA sequence
atggccagcgctcccatctccatgctggctatgctgtttaccctgagcggcctgagagcacagtctgtggctcagcccgaggaccaag
tgaatgtggccgagggcaatccccctgaccgtgaagtgcacatactcc**gtgtctggcaacccctat**ttgttttggtatgttcagtatccga
accggggcctccagttcctcctgaag**tacatcaccggcgacaatctcgtg**aagggctcctacggattcgaagccgagtttaacaag
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gttccagaagcttgtgttcggaactggcacccgacttctggtcagtccaaacATCCAGAACCCCGACCCCGCCG
TGTACCAGCTGAGGGACTCCAAGTCCAGCGACAAGAGCGTGTGTCTGTTTACGG
ACTTCGACAGCCAGACCAACGTGAGTCAAAGCAAGGACAGCGACGTCTACATAA
CGGATAAGACCGTGCTGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCG
TGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGCAT
CATCCCCGAGGACACCTTCTTCCCCAGCAGCGACGTGCCCTGCGACGTGAAACTG
GTGGAGAAGTCCTTCGAGACAGACACCAATCTGAACTTTCAGAACCTGCTGGTG
ATCGTGCTGCGGATTCTGCTGCTGAAAGTGGCCGGCTTCAATCTGCTGATGACCC
TGCGGCTGTGGAGCAGC

Alpha chain Protein sequence
MASAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPLTVKCTYS**VSGNPY**LFWYV
QYPNRGLQFLLK**YITGDNLV**KGSYGFEAEFNKSQTSFHLKKPSALVSDSALYF**CAVR**
AVNTGFQKLVFGTGTRLLVSPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQ

SKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSSDVP
CDVKLVEKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

Beta chain:
TRBV7-9/TRBJ2-3/TRBC1

Beta chain DNA sequence
atgggaacctctctgctgtgctggatggccctgtgtctgctgggagccgatcatgccgatacgggagtgtcccaggatcctcggcaca
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TCTGAAAGAACAGCCTGCCCTGAACGACAGCCGGTACTGCCTGAGCTCCAGACT
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GCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAGAGCCAAGCC
CGTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCATCAC
CAGCGCCTCCTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGAAATCCTG
CTGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGCCA
TGGTCAAGCGGAAGGACTTT

Beta chain Protein sequence
MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS**
SLEVLKETQYFGPGTRLTVLEDLNKVFPPEVAVFEPSKAEIAHTQKATLVCLATGFF
PDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH
FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGITSASYHQGVLSATILYE
ILLGKATLYAVLVSALVLMAMVKRKDF

Complete Beta and Alpha ORF DNA Sequence
atgggaacctctctgctgtgctggatggccctgtgtctgctgggagccgatcatgccgatacgggagtgtcccaggatcctcggcaca
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ATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCGGATTCTGCTGCTGAAAGT
GGCCGGCTTCAATCTGCTGATGACCCTGCGGCTGTGGAGCAGC

Complete Beta and Alpha ORF Protein Sequence
MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPISEHNRLYWYR
QTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYLCAS
SLEVLKETQYFGPGTRLTVLEDLNKVFPPEVAVFEPSKAEIAHTQKATLVCLATGFF
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RAVNTGFQKLVFGTGTRLLVSPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVS
QSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSSD
VPCDVKLVEKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

MAGEC2_184-563 wild type sequence

Alpha chain DNA sequence
TRAV3/TRAJ8/TRAC

atggcctctgcacccatctcgatgcttgcgatgctcttcacattgagtgggctgagagctcagtcagtggctcagccggaagatcaggt
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GAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGG
GTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGG
CTGTGGTCCAGC

Alpha chain Protein sequence
MASAPISMLAMLFTLSGLRAQSVAQPEDQVNVAEGNPLTVKCTYSVSGNPYLFWYV
QYPNRGLQFLLKYITGDNLVKGSYGFEAEFNKSQTSFHLKKPSALVSDSALYFCAVR
AVNTGFQKLVFGTGTRLLVSPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQ
SKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESS
CDVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Beta chain DNA sequence
TRBV7-9/TRBJ2-3/TRBC1

Atgggcaccagcctcctctgctggatggccctgtgtctcctgggggcagatcacgcagatactggagtctcccagaaccccagacac
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AGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCC
AGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCCG
TCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTC
GGTGTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTA
GGGAAGGCCACCCTGTATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGG
TCAAGAGAAAGGATTTC

Beta chain Protein sequence

MGTSLLCWMALCLLGADHADTGVSQNPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS
SLEVLKETQYF**GPGTRLTVLEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATGFFP
DHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHF
RCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEI
LLGKATLYAVLVSALVLMAMVKRKDF

MAGEC2_184-724 MGTM codon optimized sequence

Alpha chain:
TRAV19/TRAJ56/TRAC

Alpha chain DNA sequence
atgctgaccgctagcctgctgagagccgtgatcgcctctatctgtgtggtgtctagcatggcccagaaagtgacacaggcccagaccg
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AACAGCATCATCCCCGAGGACACCTTCTTCCCCAGCAGCGACGTGCCCTGCGACG
TGAAACTGGTGGAGAAGTCCTTCGAGACAGACACCAATCTGAACTTTCAGAACC
TGCTGGTGATCGTGCTGCGGATTCTGCTGCTGAAAGTGGCCGGCTTCAATCTGCT
GATGACCCTGCGGCTGTGGAGCAGC

Alpha chain Protein sequence
MLTASLLRAVIASICVVSSMAQKVTQAQTEISVVEKEDVTLDCVYE**TRDTTYY**LFW
YKQPPSGELVFLIR**RNSFDEQN**EISGRYSWNFQKSTSSFNFTITASQVVDSAVYF**CAL**
SESGANSKLTFGKGITLSVRPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQS
KDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSSDVP
CDVKLVEKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

Beta chain:
TRBV7-9/TRBJ2-3/TRBC1

Beta chain DNA sequence
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Beta chain Protein sequence
MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS**
SPRWSGGTDTQYFGPGTRLTVLEDLNKVFPPEVAVFEPSKAEIAHTQKATLVCLAT
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RNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGITSASYHQGVLSAT
ILYEILLGKATLYAVLVSALVLMAMVKRKDF

Complete Beta and Alpha ORF DNA Sequence
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Complete Beta and Alpha ORF Protein Sequence
MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPI**SEHNR**LYWYR
QTLGQGPEFLTY**FQNEAQ**LEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYL**CAS**
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F**CALSESGANSKLTF**GKGITLSVRPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTN
VSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSS
DVPCDVKLVEKSFETDTNLNFQNLLVIVLRILLLKVAGFNLLMTLRLWSS

MAGEC2_184-724 wild type sequence

Alpha chain DNA sequence
TRAV19/TRAJ56/TRAC

atgctgactgccagcctgttgagggcagtcatagcctccatctgtgttgtatccagcatggctcagaaggtaactcaagcgcagactga
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GAGAAAAGCTTTGAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATT
GGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGC
GGCTGTGGTCCAGC

Alpha chain Protein sequence

MLTASLLRAVIASICVVSSMAQKVTQAQTEISVVEKEDVTLDCVYETRDTTYYLFW
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SESGANSKLTFGKGITLSVRPDIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQS
KDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSC
DVKLVEKSFETDTNLNFQNLSVIGFRILLLKVAGFNLLMTLRLWSS

Beta chain DNA sequence
TRBV7-9/TRBJ2-3/TRBC1

Atgggcaccagcctcctctgctggatggccctgtgtctcctgggggcagatcacgcagatactggagtctcccagaaccccagacac
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Beta chain Protein sequence

MGTSLLCWMALCLLGADHADTGVSQNPRHKITKRGQNVTFRCDPISEHNRLYWYR
QTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERPKGSFSTLEIQRTEQGDSAMYLCAS
SPRWSGGTDTQYFGPGTRLTVLEDLNKVFPPEVAVFEPSEAEISHTQKATLVCLATG
FFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPR
NHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATI
LYEILLGKATLYAVLVSALVLMAMVKRKDF

* Table 2 provides, in part, representative TCR sequences grouped according to MHC serotype presentation and sub-grouped according to different peptides presented by the MHC serotype and bound by the sub-grouped TCRs. Individual TCRs, such as those representatively exemplified in the tables, are described and claimed, as well as the genus of binding proteins that bind a peptide epitope sequence described herein either alone or in a complex with an MHC, such as those grouped in the tables provided herein. In addition, TRAV, TRAJ, and TRAC genes for each TCR alpha chain described herein, and TRBV, TRBJ, and TRBC genes for each TCR beta chain described herein, are provided. Sequences for each TCR described herein are provided as pairs of cognate alpha chain and beta chains for each named TCR. TCR sequences described herein are annotated. Variable domain sequences are shown in lower case. Constant domain sequences are capitalized. CDR1,

CDR2, and CDR3 sequences are annotated using bold and underlined text. CDR1, CDR2, and CDR3 are shown in standard order of appearance from left (N-terminus) to right (C-terminus). TRAV, TRAJ, and TRAC genes for each TCR alpha chain described herein, and TRBV, TRBJ, and TRBC genes for each TCR beta chain described herein, are annotated according to well-known IMGT nomenclature described herein. Similarly, CDR1 and CDR2 of TRAV and TRBV are well-known in the art since they are based on well-known and annotated TRAV and TRBV sequences (*e.g.*, as annotated in databases like IMGT available at imt.org and IEDB available at iedb.org).

Table 3

Representative Human MAGEC2 cDNA sequence

atgcctcccgttccaggcgttccattccgcaacgttgacaacgactccccgacctcagttgagttagaagactgggtagatgcacagca
tcccacagatgaggaagaggaggaagcctcctccgcctcttccactttgtacttagtatttttcccccctcttctttctccacatcctcttctctg
attcttggtggtcctgaggaggaggaggtgccctctggtgtgataccaaatcttaccgagagcattcccagtagtcctccacagggtcct
ccacagggtccttcccagagtcctctgagctcctgctgctcctcttttttcatggagctcattcagtgaggagtccagcagccagaaaggg
gaggatacaggcacctgtcagggcctgccagacagtgagtcctctttcacatatacactagatgaaaaggtggccgagttagtggagt
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tactcaagagagcccgtgagttcatggagcttctttttggccttgccctgatagaagtgggccctgaccacttctgtgtgtttgcaaacac
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gcaactgtgcctctgaggaggtcatctgggaagtgctgaatgcagtaggggtatatgctgggagggagcacttcgtctatggggagc
ctagggagctcctcactaaagtttgggtgcagggacattacctggagtatcgggaggtgccccacagttctcctccatattatgaattcct
gtggggtccgagagcccatttcagaaagcatcaagaagaaagtactagagttttttagccaagctgaacaacactgttcctagttcctttcc
atcctggtacaaggatgctttgaaagatgtggaagagagagtccaggccacaattgataccgcagatgatgccactgtcatggccagt
gaaagcctcagtgtcatgtccagcaacgtctccttttctgagtga

Representative Human MAGEC2 protein sequence (Representative, non-limiting epitopes underlined)

MPPVPGVPFRNVDNDSPTSVELEDWVDAQHPTDEEEEEASSASSTLYLVFSPSSFSTS
SSLILGGPEEEEVPSGVIPNLTESIPSSPPQGPPQGPSQSPLSSCCSSFSWSSFSEESSSQK
GEDTGTCQGLPDSESSFTYTLDEKVAELVEFLLLKYEAEEPVTEAEMLMIVIKYKDYF
PVILKRAREFMELLFGLALIEVGPDHFCVFANTVGLTDEGSDDEGMPENSLLIIILSVIF
IKGNCASEEVIWEVLNAVGVYAGREHFVYGEPRELLTKVWVQGHYLEYREVPHSSP
PYYEFLWGPRAHSESIKKKVLEFLAKLNNTVPSSFPSWYKDALKDVEERVQATIDTA
DDATVMASESLSVMSSNVSFSE

Representative Human HLA-B*07:02 DNA sequence

atgctggtcatggcgccccgaaccgtcctcctgctgctctcggcggccctggccctgaccgagacctgggccggctcccactccatg
aggtatttctacacctccgtgtcccggccccggccgcggggagccccgcttcatctcagtgggctacgtggacgacacccagttcgtga
ggttcgacagcgacgccgcgagtccgagagaggagccgcgggcgccgtggatagagcaggaggggccggagtattgggaccg
gaacacacagatctacaaggcccaggcacagactgaccgagagagcctgcggaacctgcgcggctactacaaccagagcgaggc
cgggtctcacaccctccagagcatgtacggctgcgacgtggggccggacgggcgcctcctccgcgggcatgaccagtacgcctac
gacggcaaggattacatcgccctgaacgaggacctgcgctcctggaccgccgcggcacacggcggctcagatcacccagcgcaagt
gggaggcggcccgtgaggcggagcagcggagagcctacctggagggcgagtgcgtggagtggctccgcagatacctggagaac
gggaaggacaagctggagcgcgctgacccccccaaagacacacgtgacccaccaccccatctctgaccatgaggccaccctgaggt
gctgggccctgggttctaccctgcggagatcacactgacctggcagcgggatggcgaggaccaaactcaggacactgagcttgtg

gagaccagaccagcaggagatagaaccttccagaagtgggcagctgtggtggtgccttctggagaagagcagagatacacatgcca
tgtacagcatgaggggctgccgaagccccteaccctgagatgggagccgtcttcccagtccaccgtccccatcgtgggcattgttgct
ggcctggctgtcctagcagttgtggtcatcggagctgtggtcgctgctgtgatgtgtaggaggaagagttcaggtggaaaaggaggg
agctactctcaggctgcgtgcagcgacagtgcccagggctctgatgtgtctctcacagcttga

Representative Human HLA-B*07:02 protein sequence

MLVMAPRTVLLLLSAALALTETWAGSHSMRYFYTSVSRPGRGEPRFISVGYVDDTQ
FVRFDSDAASPREEPRAPWIEQEGPEYWDRNTQIYKAQAQTDRESLRNLRGYYNQSE
AGSHTLQSMYGCDVGPDGRLLRGHDQYAYDGKDYIALNEDLRSWTAADTAAQITQ
RKWEAAREAEQRRAYLEGECVEWLRRYLENGKDKLERADPPKTHVTHHPISDHEAT
LRCWALGFYPAEITLTWQRDGEDQTQDTELVETRPAGDRTFQKWAAVVVPSGEEQR
YTCHVQHEGLPKPLTLRWEPSSQSTVPIVGIVAGLAVLAVVVIGAVVAAVMCRRKSS
GGKGGSYSQAACSDSAQGSDVSLTA

Representative Vector: pNVVD187_TSC-201-B07_TCR-6_MSCV-TCR-6-CD8-EF1α-dnTGFbRII-DHFR

GCTAGCTGGCTTGTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAAGCCTTAT
ATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGAT
TTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCT
TAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAA
ACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTT
GAACTGCTGATCTGTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAATATTGAGT
TCGCACATCTTGTTGTCTGATTATTGATTTTTGGCGAAACCATTTGATCATATGAC
AAGATGTGTATCTACCTTAACTTAATGATTTTGATAAAAATCATTAGGTACCAAT
TACATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGAAA
GATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGATTC
AATTTTTTTTAAACTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTTGA
GAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAATCT
CACATAATAACTTTATTTCAATCACACAATTGAATACCAATAGGTTGACAGTACT
TACCAGCCTGCAGG**TGAAAGACCCCACCTGTAGGTTTGGCAAGTTAGCTTAA**
GTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATA
TCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCC
CAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATCAGATGTTTCCAG
GGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCA
GTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAGCTCAATAAAAG
AGCCCACAACCCCTCACTTGGTGGGCCAGTCCTCTGATAGACTGTGTCCCCT
GGATACCCGTACGGTACCGCTAGCGCCACC*ATGGGAACCTCTCTGCTGTGCTGGA*
TGGCCCTGTGTCTGCTGGGAGCCGATCATGCCGATACGGGAGTGTCCCAGGATCC
TCGGCACAAGATTACCAAGAGGGGCCAGAACGTGACCTTCCGCTGTGACCCTATCA
GCGAGCACAACCGGCTGTATTGGTACAGACAGACACTCGGCCAGGGGCCTGAATT
TCTCACATACTTCCAGAACGAAGCCCAGCTGGAAAAGTCCCGGCTGCTGAGCGATA
GATTTTCCGCCGAGAGGCCCAAGGGCTCCTTCAGCACTCTGGAAATTCAGCGCACC
GAGCAGGGCGACTCTGCCATGTATCTGTGCGCATCCTCTAAGGGCATTTTTGAGCA
ATATTTTGGGCCGGGCACCAGGCTCACGGTCACAGAAGATCTGAACAAGGTGTTCC
CTCCAGAGGTGGCCGTGTTCGAGCCTTCTAAGGCCGAGATCGCCCACACACAAAAA
GCCACCCTCGTGTGCCTGGCCACCGGCTTTTTCCCCGACCACGTGGAACTGTCTTG
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AAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCATCACCAGCGCCTC
CTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGAAATCCTGCTGGGCAAG
GCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGCCATGGTCAAGCG
*GAAGGACTTT*GGCAGCGGCAGAGCCAAAAGGTCCGGGAGCGGTGCGACAAACTT
TAGCCTGTTGAAACAAGCCGGCGACGTTGAAGAGAACCCCGGACCT**ATGAAGAG**
AATCCTGGGCGCCCTGCTGGGACTGCTGTCTGCCCAAGTCTGTTGTGTGCG
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CAACAGCACCCTGAGATGCAACTTCAGCGACTCCGTGAACAACCTGCAGTG
GTTCCACCAGAATCCTTGGGGCCAGCTGATCAACCTGTTCTACATCCCCAGC
GGCACCAAGCAGAACGGCAGACTGTCTGCTACCACCGTGGCCACCGAGAGA
TACAGCCTGCTGTACATCAGCAGCAGCCAGACCACCGATTCCGGCGTCTACT
TCTGCGCAGTGACTGGTACCGCTTCAAAACTGACATTTGGGACTGGAACAA
GACTTCAGGTCACGCTCGACATCCAGAACCCCGACCCCGCCGTGTACCAGC
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ACAGCCAGACCAACGTGAGTCAAAGCAAGGACAGCGACGTCTACATAACGG
ATAAGACCGTGCTGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCG
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GCATCATCCCCGAGGACACCTTCTTCCCCAGCAGCGACGTGCCCTGCGACG
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TCTGCTGATGACCCTGCGGCTGTGGAGCAGCAGGGCTAAGAGGTCCGGCAGC
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GGCCCC*ATGGCGCTGCCCGTCACCGCGCTGCTGCTGCCCCTGGCGCTGCTGTTACAC*
GCCGCTCGGCCA<u>*GAGCTTCCCACCCAGGGCACATTCTCCAACGTGTCCACCAATGTGT*</u>
<u>*CG*</u>*GGAGGCGGCGGATCGTCCCAGTTCAGAGTGTCCCCTCTGGACCGCACCTGGAACC*
TGGGCGAGACCGTGGAGCTGAAATGTCAGGTCCTGCTGAGCAACCCGACCTCCGGGT
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GAGCCAGAACAAGCCCAAGGCGGCCGAGGGCCTGGACACCCAGAGATTCTCCGGCA
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CTCCGTTCTGCAGCAGACTCCCGCCTACATCAAGGTGCAGACGAACAAGATGGT
GATGCTGTCATGCGAGGCCAAGATCTCTCTTTCAAATATGAGAATTTATTGGCTA
CGACAGCGCCAGGCCCCCTCCAGCGACAGCCACCACGAGTTCCTGGCGCTTTGG
GATTCTGCTAAAGGCACCATCCATGGAGAGGAGGTGGAACAGGAGAAGATAGCT
GTCTTCCGCGACGCATCCCGCTTCATCCTGAACCTGACCAGCGTGAAGCCGGAGG
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GAAATCCACCCTCAAGAAGCGCGTGTGCCGACTGCCCCGCCCTGAAACCCAGAA
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CTGCTCGTATCTCTGGGTGTCGCCATCCACCTGTGCTGCCGCCGCCGCCGCGCCC
GCCTGAGGTTTATGAAACAGTTTTACAAGTGATAAATCGATGGAAGGGTGGCAT

CCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTGGAAGTTGCCACTCCAGTGC
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TTCTATAATATTATGGGGTGGAGGGGGGTGGTATGGAGCAAGGGGCAAGTTGGG
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GCACAATCTTGGCTCACTGCAATCTCCGCCTCCTGGGTTCAAGCGATTCTCCTGCC
TCAGCCTCCCGAGTTGTTGGGATTCCAGGCATGCATGACCAGGCTCAGCTAATTT
TTGTTTTTTTGGTAGAGACGGGGTTTCACCATATTGGCCAGGCTGGTCTCCAACTC
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TGAACCACTGCTCCCTTCCCTGTCCTTCTGATTACTAGTGGCTCCGGTGCCCGTCA
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GTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAA
GTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGT
GCCTTGAATTACTTCCACCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGG
GTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTC
GTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTG
GCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTT
GATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCA
AGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGT
GCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGA
ATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGC
CGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGC
GTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAG
GACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGG
CCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTC
CAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGG
GAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTA
GGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA
TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCA
GGTGTCGTGAACTAGTCCAGTGTGGTGGAATTCTGCAGATATCACGGCTAGCGCC
ACCATGGGTCGGGGGCTGCTCAGGGGCCTGTGGCCGCTGCACATCGTCCTGTGGA
CGCGTATCGCCAGCACGATCCCACCGCACGTTCAGAAGTCGGTGAATAACGACA
TGATAGTCACTGACAACAACGGTGCAGTCAAGTTTCCACAACTGTGTAAATTTTG
TGATGTGAGATTTTCCACCTGTGACAACCAGAAATCCTGCATGAGCAACTGCAGC
ATCACCTCCATCTGTGAGAAGCCACAGGAAGTCTGTGTGGCTGTATGGAGAAAG
AATGACGAGAACATAACACTAGAGACAGTTTGCCATGACCCCAAGCTCCCCTAC
CATGACTTTATTCTGGAAGATGCTGCTTCTCCAAAGTGCATTATGAAGGAGAAGA
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CAACATCATCTTCTCAGAAGAATATAACACCAGCAATCCTGACTTGTTGCTAGTC
ATATTTCAAGTGACAGGCATCAGCCTCCTGCCACCACTGGGAGTTGCCATATCTG
TCATCATCATCTTCTACTGCTACCGCGTGAACCGGCAGCAGAAGGCTAGTGGTTC
AGGCGCAACGAATTTCTCTTTGCTGAAGCAGGCTGGGGATGTCGAAGAAAATCC
GGGTCCAATGGTGGGCTCGCTCAACTGCATCGTAGCAGTCTCCCAGAATATGGGC
ATCGGGAAGAACGGTGATTTCCCGTGGCCCCCACTTCGCAACGAGAGCCGTTATT
TCCAAAGAATGACTACAACCTCCTCCGTGGAGGGTAAGCAGAACCTGGTCATCA
TGGGGAAGAAGACCTGGTTCTCTATCCCTGAAAAAAACCGCCCCCTGAAGGGCC
GCATCAACCTGGTGCTGAGCAGGGAACTCAAGGAGCCTCCTCAGGGCGCGCATT
TTCTGAGCCGCTCATTGGATGACGCTCTCAAACTGACCGAACAGCCGGAGCTAGC
CAACAAGGTGGACATGGTGTGGATCGTCGGAGGCTCCTCCGTGTACAAGGAGGC

CATGAATCACCCCGGCCACTTGAAGCTGTTCGTCACCCGGATCATGCAGGACTTC
GAGTCGGACACGTTCTTTCCAGAGATTGACCTGGAGAAGTACAAGCTGCTGCCCG
AGTACCCGGGAGTTCTTAGTGATGTGCAGGAGGAGAAAGGCATCAAGTACAAAT
TTGAGGTGTACGAGAAGAACGACTAACGGTCCGTCCTGACCAATGCTGGAGTTCT
TCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAG
CATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGT
CCAAACTCATCAATGTATCTTATCATGTCTGTATACAGGTTACCTCAGTCTCCTAG
GTACGTCTTATATCTATGAAAAAACATTCAAAAGCACAACATCTAGAAGAACTTA
CCTTTTTTCACCACTCTATTGCAAAGATATGTACCGATTTCTCTCGAAGTACAAAA
AACCGCTAGTTTTCAAATTCACCTCAAGACTTTGAAAAAAAATTGAATCTGTCAA
TGTCAAATAAAATCAGAAACAAATGTCATAATGTTACGTTAATGTTGTCAGGTCG
AAAAATAAAATTGCAAATAGAAATTTTGTTCCTTTTTTATTGGTTTTTATTGGTGG
GAAAAATATTCCCTCTAACTGCAAAAGGGTTAATTATGTTAGAGGTAGAGTCGAC

* For certain depicted vectors, MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

Representative Vector pNVVD188_TCR-422_MSCV-TCR- 422-CD8-EF1α-dnTGFbRII-DHFR

GCTAGCTGGCTTGTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAAGCCTTAT
ATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGAT
TTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCT
TAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAA
ACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTT
GAACTGCTGATCTGTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAATATTGAGT
TCGCACATCTTGTTGTCTGATTATTGATTTTTGGCGAAACCATTTGATCATATGAC
AAGATGTGTATCTACCTTAACTTAATGATTTTGATAAAAATCATTAGGTACCAAT
TACATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGAAA
GATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGATTC
AATTTTTTTTTAAACTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTTGA
GAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAATCT
CACATAATAACTTTATTTCAATCACACAATTGAATACCAATAGGTTGACAGTACT
TACCAGCCTGCAGG**TGAAAGACCCCACCTGTAGGTTTGGCAAGTTAGCTTAA**
GTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATA
TCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCC
CAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATCAGATGTTTCCAG
GGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCA
GTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAGCTCAATAAAAG
AGCCCACAACCCCTCACTTGGTGGGCCAGTCCTCTGATAGACTGTGTCCCCT
GGATACCCGTACGGTACCGCTAGCGCCACC***ATGGGCCCTGGACTGCTGCATTGGA***
TGGCTCTGTGCCTGCTCGGAACAGGACATGGCGACGCTATGGTCATTCAGAACCCC
AGATACCAAGTGACACAGTTCGGCAAGCCCGTGACTCTGAGCTGCTCTCAGACCCT
GAACCACAACGTGATGTATTGGTATCAGCAGAAGTCCTCTCAGGCCCCTAAGCTGC
TGTTCCACTACTACGACAAGGACTTCAACAACGAGGCCGACACACCCGACAACTTC

CAGAGCAGAAGGCCCAATACCAGCTTCTGCTTCCTGGACATCAGAAGCCCAGGACT
GGGAGATGCCGCCATGTATCTGTGCGCTACTTCTGGGGGGGGCCAAGGGCTCCTC
TCAAGCTACAATGAACAATTCTTCGGGCCAGGGACACGGCTCACCGTGCTAGAAGA
TCTGAACAAGGTGTTCCCTCCAGAGGTGGCCGTGTTCGAGCCTTCTAAGGCCGAGA
TCGCCCACACACAAAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTTCCCCGAC
CACGTGGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACTCCGGCGTGTCAA
CGGATCCCCAGCCTCTGAAAGAACAGCCTGCCCTGAACGACAGCCGGTACTGCCT
GAGCTCCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCACTTCA
GATGCCAGGTGCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAG
AGCCAAGCCCGTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGC
GGCATCACCAGCGCCTCCTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGA
AATCCTGCTGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGA
*TGGCCATGGTCAAGCGGAAGGACTTT*GGCAGCGGCAGAGCCAAAAGGTCCGGGA
GCGGTGCGACAAACTTTAGCCTGTTGAAACAAGCCGGCGACGTTGAAGAGAACC
CCGGACCT**ATGACCAGCATCCGGGCCGTGTTCATCTTCCTCTGGCTCCAACT**
GGACCTCGTGAACGGCGAGAACGTGGAACAGCATCCCTCCACACTGTCAGT
GCAAGAGGGCGACTCTGCCGTGATCAAGTGCACATACAGTGACTCCGCCAG
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GCAAGGACAGCGACGTCTACATAACGGATAAGACCGTGCTGGACATGCGGA
GCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACT
TCGCCTGCGCCAACGCCTTCAACAACAGCATCATCCCCGAGGACACCTTCTT
CCCCAGCAGCGACGTGCCCTGCGACGTGAAACTGGTGGAGAAGTCCTTCGA
GACAGACACCAATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCGGATT
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*TGCTGCTGCCCCTGGCGCTGCTGTTACACGCCGCTCGGCCA*GAGCTTCCCACCCAGG
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TTCCATCATGTATTTCAGCCACTTTGTGCCAGTGTTCCTGCCTGCCAAGCCTACCACAA
CACCAGCTCCCCGTCCCCCGACTCCGGCGCCTACCATCGCGAGTCAACCGTTGAGCC
TGAGGCCTGAGGCTTGTCGGCCCGCTGCGGGGGGTGCCGTCCACACCAGGGGCCTC
GACTTTGCGTGCGACATCTATATTTGGGCGCCTCTGGCGGGTACCTGCGGGGTGCTG
CTGCTGTCATTGGTGATTACCCTGTACTGCAATCACCGCAACCGCCGGCGGGTCTGTA
AGTGCCCACGGCCTGTGGTCAAGTCCGGTGACAAACCGTCGCTCTCGGCTCGCTACG
*TG*CGCGCTAAGCGCAGCGGTTCCGGGGCCACCAACTTTTCATTGCTGAAGCAGGC
CGGTGATGTGGAGGAGAATCCAGGGCCC**ATGCGCCCCAGGCTTTGGCTCCTTCTT**
GCTGCTCAGCTCACTGTCTTGCATGGCAACTCCGTTCTGCAGCAGACTCCCGCCT
ACATCAAGGTGCAGACGAACAAGATGGTGATGCTGTCATGCGAGGCCAAGATCT
CTCTTTCAAATATGAGAATTTATTGGCTACGACAGCGCCAGGCCCCCTCCAGCGA

CAGCCACCACGAGTTCCTGGCGCTTTGGGATTCTGCTAAAGGCACCATCCATGGA
GAGGAGGTGGAACAGGAGAAGATAGCTGTCTTCCGCGACGCATCCCGCTTCATC
CTGAACCTGACCAGCGTGAAGCCGGAGGACAGCGGCATCTACTTCTGTATGATC
GTTGGCTCCCCCGAGCTGACCTTCGGCAAAGGCACCCAGCTGTCCGTGGTGGACT
TCCTGCCCACCACAGCCCAGCCAACCAAGAAATCCACCCTCAAGAAGCGCGTGT
GCCGACTGCCCCGCCCTGAAACCCAGAAGGGCCCTCTGTGCTCCCCCATCACCCT
TGGACTGCTGGTGGCGGGAGTCCTGGTGCTGCTCGTATCTCTGGGTGTCGCCATC
CACCTGTGCTGCCGCCGCCGCCGCGCCCGCCTGAGGTTTATGAAACAGTTTTACA
AGTGATAAATCGATGGAAGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCTCTCC
TGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAAATTAAG
TTGCATCATTTTGTCTGACTAGGTGTCCTTCTATAATATTATGGGGTGGAGGGGG
GTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGGGGTC
TATTGGGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATCTCCG
CCTCCTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCCGAGTTGTTGGGATTCCAG
GCATGCATGACCAGGCTCAGCTAATTTTTGTTTTTTTGGTAGAGACGGGGTTTCA
CCATATTGGCCAGGCTGGTCTCCAACTCCTAATCTCAGGTGATCTACCCACCTTG
GCCTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGTCCTTC
TGATTACTAGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGT
CCCCGAGAAGTTGTGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTG
GCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAG
GGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCA
ACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTG
GCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAG
TACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGC
CTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCG
CTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTC
GATAAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTG
GCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTT
GGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGG
CGGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGC
CGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGG
CAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCG
GCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCG
GGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCAT
GTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTT
TTGGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCC
CACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCT
CCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACA
GTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAACTAGTCCAGTGTGGTG
GAATTCTGCAGATATCACGGCTAGCGCCACCATGGGTCGGGGGCTGCTCAGGGG
CCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGCACGATCCCACCG
CACGTTCAGAAGTCGGTGAATAACGACATGATAGTCACTGACAACAACGGTGCA
GTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACA
ACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCAC
AGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGA
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TGTTCCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATA
ACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAAGTGACAGGCATCAGCCT
CCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCG

TGAACCGGCAGCAGAAGGCTAGTGGTTCAGGCGCAACGAATTTCTCTTTGCTGAA
GCAGGCTGGGGATGTCGAAGAAAATCCGGGTCCAATGGTGGGCTCGCTCAACTG
CATCGTAGCAGTCTCCCAGAATATGGGCATCGGGAAGAACGGTGATTTCCCGTG
GCCCCCACTTCGCAACGAGAGCCGTTATTTCCAAAGAATGACTACAACCTCCTCC
GTGGAGGGTAAGCAGAACCTGGTCATCATGGGGAAGAAGACCTGGTTCTCTATC
CCTGAAAAAAACCGCCCCCTGAAGGGCCGCATCAACCTGGTGCTGAGCAGGGAA
CTCAAGGAGCCTCCTCAGGGCGCGCATTTTCTGAGCCGCTCATTGGATGACGCTC
TCAAACTGACCGAACAGCCGGAGCTAGCCAACAAGGTGGACATGGTGTGGATCG
TCGGAGGCTCCTCCGTGTACAAGGAGGCCATGAATCACCCCGGCCACTTGAAGCT
GTTCGTCACCCGGATCATGCAGGACTTCGAGTCGGACACGTTCTTTCCAGAGATT
GACCTGGAGAAGTACAAGCTGCTGCCCGAGTACCCGGGAGTTCTTAGTGATGTG
CAGGAGGAGAAAGGCATCAAGTACAAATTTGAGGTGTACGAGAAGAACGACTA
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GCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCA
TTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCAT
GTCTGTATACAGGTTACCTCAGTCTCCTAGGTACGTCTTATATCTATGAAAAAAC
ATTCAAAAGCACAACATCTAGAAGAACTTACCTTTTTTCACCACTCTATTGCAAA
GATATGTACCGATTTCTCTCGAAGTACAAAAAACCGCTAGTTTTCAAATTCACCT
CAAGACTTTGAAAAAAAATTGAATCTGTCAATGTCAAATAAAATCAGAAACAAA
TGTCATAATGTTACGTTAATGTTGTCAGGTCGAAAAATAAAATTGCAAATAGAAA
TTTTGTTCCTTTTTTATTGGTTTTTATTGGTGGGAAAAATATTCCCTCTAACTGCAA
AAGGGTTAATTATGTTAGAGGTAGAGTCGAC

* For certain depicted vectors, MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

Representative Vector pNVVD189_TCR-724_MSCV-TCR- 724-CD8-EF1α-dnTGFbRII-DHFR

GCTAGCTGGCTTGTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAAGCCTTAT
ATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGAT
TTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCT
TAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAA
ACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTT
GAACTGCTGATCTGTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAATATTGAGT
TCGCACATCTTGTTGTCTGATTATTGATTTTTGGCGAAACCATTTGATCATATGAC
AAGATGTGTATCTACCTTAACTTAATGATTTTGATAAAAATCATTAGGTACCAAT
TACATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGAAA
GATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGATTC
AATTTTTTTTTAAACTTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTTGA
GAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAATCT
CACATAATAACTTTATTTCAATCACACAATTGAATACCAATAGGTTGACAGTACT
TACCAGCCTGCAGG**TGAAAGACCCCACCTGTAGGTTTGGCAAGTTAGCTTAA**
GTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATA
TCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCC
CAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATCAGATGTTTCCAG

GGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCA
GTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAGCTCAATAAAAG
AGCCCACAACCCCTCACTTGGTGGGCCAGTCCTCTGATAGACTGTGTCCCCT
GGATACCCGTACGGTACCGCTAGCGCCACC*ATGGGAACCTCTCTGCTGTGCTGGA*
TGGCCCTGTGTCTGCTGGGAGCCGATCATGCCGATACGGGAGTGTCCCAGGATCC
TCGGCACAAGATTACCAAGAGGGGCCAGAACGTGACCTTCCGCTGTGACCCTATCA
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GCACTGACACACAGTACTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAAGATCTG
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TGGAACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACTCCGGCGTGTCAACGGA
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CCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCACTTCAGATGC
CAGGTGCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAGAGCCA
AGCCCGTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCAT
CACCAGCGCCTCCTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGAAATCC
TGCTGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGC
*CATGGTCAAGCGGAAGGACTTT*GGCAGCGGCAGAGCCAAAAGGTCCGGGAGCGG
TGCGACAAACTTTAGCCTGTTGAAACAAGCCGGCGACGTTGAAGAGAACCCCGG
ACCT**ATGCTGACCGCTAGCCTGCTGAGAGCCGTGATCGCCTCTATCTGTGTG**
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GCTGGAACTTCCAGAAGTCCACCAGCAGCTTCAACTTCACCATCACCGCCAG
CCAGGTGGTGGATAGCGCCGTGTACTTCTGCGCACTTTCCGAAAGCGGGGC
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GACAAGAGCGTGTGTCTGTTTACGGACTTCGACAGCCAGACCAACGTGAGT
CAAAGCAAGGACAGCGACGTCTACATAACGGATAAGACCGTGCTGGACATG
CGGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGC
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TCTTCCCCAGCAGCGACGTGCCCTGCGACGTGAAACTGGTGGAGAAGTCCT
TCGAGACAGACACCAATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCG
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GAAACAGGCTGGTGACGTGGAAGAAAACCCTGGCCCC*ATGGCGCTGCCCGTCACC*
GCGCTGCTGCTGCCCCTGGCGCTGCTGTTACACGCCGCTCGGCCA<u>*GAGCTTCCCACC*</u>
<u>*CAGGGCACATTCTCCAACGTGTCCACCAATGTGTCG*</u>*GGAGGCGGCGGATCGTCCCAG*
TTCAGAGTGTCCCCTCTGGACCGCACCTGGAACCTGGGCGAGACCGTGGAGCTGAAA
TGTCAGGTCCTGCTGAGCAACCCGACCTCCGGGTGCAGTTGGCTGTTCCAGCCGCGT
GGTGCTGCCGCAAGCCCTACGTTCCTGCTTTACCTGAGCCAGAACAAGCCCAAGGCG
GCCGAGGGCCTGGACACCCAGAGATTCTCCGGCAAGCGCCTGGGGGACACATTCGTG
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ACAACACCAGCTCCCCGTCCCCCGACTCCGGCGCCTACCATCGCGAGTCAACCGTTG
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AGGCCGGTGATGTGGAGGAGAATCCAGGGCCCATGCGCCCCAGGCTTTGGCTCC
TTCTTGCTGCTCAGCTCACTGTCTTGCATGGCAACTCCGTTCTGCAGCAGACTCCC
GCCTACATCAAGGTGCAGACGAACAAGATGGTGATGCTGTCATGCGAGGCCAAG
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GCGACAGCCACCACGAGTTCCTGGCGCTTTGGGATTCTGCTAAAGGCACCATCCA
TGGAGAGGAGGTGGAACAGGAGAAGATAGCTGTCTTCCGCGACGCATCCCGCTT
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ACTTCCTGCCCACCACAGCCCAGCCAACCAAGAAATCCACCCTCAAGAAGCGCG
TGTGCCGACTGCCCCGCCCTGAAACCCAGAAGGGCCCTCTGTGCTCCCCCATCAC
CCTTGGACTGCTGGTGGCGGGAGTCCTGGTGCTGCTCGTATCTCTGGGTGTCGCC
ATCCACCTGTGCTGCCGCCGCCGCCGCGCCCGCCTGAGGTTTATGAAACAGTTTT
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GGGGTGGTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGG
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TTCACCATATTGGCCAGGCTGGTCTCCAACTCCTAATCTCAGGTGATCTACCCAC
CTTGGCCTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGT
CCTTCTGATTACTAGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCC
ACAGTCCCCGAGAAGTTGTGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGA
AGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTC
CCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTT
TCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGG
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GGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTG
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TTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGG
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TGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGG
GCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTT
CCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG
GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTT
CATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAG
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CCCCACACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAAT
TCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAG
ACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAACTAGTCCAGTGTG
GTGGAATTCTGCAGATATCACGGCTAGCGCCACCATGGGTCGGGGGCTGCTCAG
GGGCCTGTGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGCACGATCCCA
CCGCACGTTCAGAAGTCGGTGAATAACGACATGATAGTCACTGACAACAACGGT

GCAGTCAAGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTG
ACAACCAGAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGC
CACAGGAAGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAG
AGACAGTTTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGC
TGCTTCTCCAAAGTGCATTATGAAGGAGAAGAAAAAGCCTGGTGAGACTTTCTTC
ATGTGTTCCTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAGAAGAAT
ATAACACCAGCAATCCTGACTTGTTGCTAGTCATATTTCAAGTGACAGGCATCAG
CCTCCTGCCACCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGCTACC
GCGTGAACCGGCAGCAGAAGGCTAGTGGTTCAGGCGCAACGAATTTCTCTTTGCT
GAAGCAGGCTGGGGATGTCGAAGAAAATCCGGGTCCAATGGTGGGCTCGCTCAA
CTGCATCGTAGCAGTCTCCCAGAATATGGGCATCGGGAAGAACGGTGATTTCCCG
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CCGTGGAGGGTAAGCAGAACCTGGTCATCATGGGGAAGAAGACCTGGTTCTCTA
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AACTCAAGGAGCCTCCTCAGGGCGCGCATTTTCTGAGCCGCTCATTGGATGACGC
TCTCAAACTGACCGAACAGCCGGAGCTAGCCAACAAGGTGGACATGGTGTGGAT
CGTCGGAGGCTCCTCCGTGTACAAGGAGGCCATGAATCACCCCGGCCACTTGAA
GCTGTTCGTCACCCGGATCATGCAGGACTTCGAGTCGGACACGTTCTTTCCAGAG
ATTGACCTGGAGAAGTACAAGCTGCTGCCCGAGTACCCGGGAGTTCTTAGTGATG
TGCAGGAGGAGAAAGGCATCAAGTACAAATTTGAGGTGTACGAGAAGAACGACT
AACGGTCCGTCCTGACCAATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGC
AGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGC
ATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATC
ATGTCTGTATACAGGTTACCTCAGTCTCCTAGGTACGTCTTATATCTATGAAAAA
ACATTCAAAAGCACAACATCTAGAAGAACTTACCTTTTTTCACCACTCTATTGCA
AAGATATGTACCGATTTCTCTCGAAGTACAAAAAACCGCTAGTTTTCAAATTCAC
CTCAAGACTTTGAAAAAAAATTGAATCTGTCAATGTCAAATAAAATCAGAAACA
AATGTCATAATGTTACGTTAATGTTGTCAGGTCGAAAAATAAAATTGCAAATAGA
AATTTTGTTCCTTTTTTATTGGTTTTTATTGGTGGGAAAAATATTCCCTCTAACTGC
AAAAGGGTTAATTATGTTAGAGGTAGAGTCGAC

* For certain depicted vectors, MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

Representative Vector pNVVD190_TCR-563_MSCV-TCR- 563-CD8-EF1α-dnTGFbRII-DHFR

GCTAGCTGGCTTGTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAAGCCTTAT
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TTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCT
TAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAA
ACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTT
GAACTGCTGATCTGTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAATATTGAGT
TCGCACATCTTGTTGTCTGATTATTGATTTTTGGCGAAACCATTTGATCATATGAC
AAGATGTGTATCTACCTTAACTTAATGATTTTGATAAAAATCATTAGGTACCAAT
TACATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGAAA

GATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGATTC
AATTTTTTTTTAAACTTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTTGA
GAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAATCT
CACATAATAACTTTATTTCAATCACACAATTGAATACCAATAGGTTGACAGTACT
TACCAGCCTGCAGGTGAAAGACCCCACCTGTAGGTTTGGCAAGTTAGCTTAA
GTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATA
TCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCC
CAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATCAGATGTTTCCAG
GGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCA
GTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAGCTCAATAAAAG
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GGATACCCGTACGGTACCGCTAGCGCCACC*ATGGGAACCTCTCTGCTGTGCTGGA*
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TCGGCACAAGATTACCAAGAGGGGCCAGAACGTGACCTTCCGCTGTGACCCTATCA
GCGAGCACAACCGGCTGTATTGGTACAGACAGACACTCGGCCAGGGGCCTGAATT
TCTCACATACTTCCAGAACGAAGCCCAGCTGGAAAAGTCCCGGCTGCTGAGCGATA
GATTTTCCGCCGAGAGGCCCAAGGGCTCCTTCAGCACTCTGGAAATTCAGCGCACC
GAGCAGGGCGACTCTGCCATGTATCTGTGCGCCTCTAGTTTGGAAGTTCTTAAGGA
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GCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAGAGCCAAGCCC
GTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCATCACCA
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GGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGCCATGG
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GAGAGCACAGTCTGTGGCTCAGCCCGAGGACCAAGTGAATGTGGCCGAGGG
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ACAGCGACGTCTACATAACGGATAAGACCGTGCTGGACATGCGGAGCATGG
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GCGCCAACGCCTTCAACAACAGCATCATCCCCGAGGACACCTTCTTCCCCAG
CAGCGACGTGCCCTGCGACGTGAAACTGGTGGAGAAGTCCTTCGAGACAGA
CACCAATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCGGATTCTGCTG
CTGAAAGTGGCCGGCTTCAATCTGCTGATGACCCTGCGGCTGTGGAGCAGC
AGGGCTAAGAGGTCCGGCAGCGGAGCCACCAATTTTTCCCTGCTGAAACAGGCT
GGTGACGTGGAAGAAAACCCTGGCCCC*ATGGCGCTGCCCGTCACCGCGCTGCTGC*
*TGCCCCTGGCGCTGCTGTTACACGCCGCTCGGCCA*GAGCTTCCCACCCAGGGCACAT

TCTCCAACGTGTCCACCAATGTGTCGGGAGGCGGCGGATCGTCCCAGTTCAGAGTGT
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TGCTGAGCAACCCGACCTCCGGGTGCAGTTGGCTGTTCCAGCCGCGTGGTGCTGCCG
CAAGCCCTACGTTCCTGCTTTACCTGAGCCAGAACAAGCCCAAGGCGGCCGAGGGCC
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CGATTTCCGCAGAGAGAACGAGGGCTACTATTTCTGTTCGGCGCTGAGCAATTCCATC
ATGTATTTCAGCCACTTTGTGCCAGTGTTCCTGCCTGCCAAGCCTACCACAACACCAGC
TCCCCGTCCCCCGACTCCGGCGCCTACCATCGCGAGTCAACCGTTGAGCCTGAGGCC
TGAGGCTTGTCGGCCCGCTGCGGGGGGTGCCGTCCACACCAGGGGCCTCGACTTTG
CGTGCGACATCTATATTTGGGCGCCTCTGGCGGGTACCTGCGGGGTGCTGCTGCTGT
CATTGGTGATTACCCTGTACTGCAATCACCGCAACCGCCGGCGGGTCTGTAAGTGCCC
ACGGCCTGTGGTCAAGTCCGGTGACAAACCGTCGCTCTCGGCTCGCTACGTGCGCGC
TAAGCGCAGCGGTTCCGGGGCCACCAACTTTTCATTGCTGAAGCAGGCCGGTGAT
GTGGAGGAGAATCCAGGGCCCATGCGCCCCAGGCTTTGGCTCCTTCTTGCTGCTC
AGCTCACTGTCTTGCATGGCAACTCCGTTCTGCAGCAGACTCCCGCCTACATCAA
GGTGCAGACGAACAAGATGGTGATGCTGTCATGCGAGGCCAAGATCTCTCTTTCA
AATATGAGAATTTATTGGCTACGACAGCGCCAGGCCCCCTCCAGCGACAGCCAC
CACGAGTTCCTGGCGCTTTGGGATTCTGCTAAAGGCACCATCCATGGAGAGGAG
GTGGAACAGGAGAAGATAGCTGTCTTCCGCGACGCATCCCGCTTCATCCTGAACC
TGACCAGCGTGAAGCCGGAGGACAGCGGCATCTACTTCTGTATGATCGTTGGCTC
CCCCGAGCTGACCTTCGGCAAAGGCACCCAGCTGTCCGTGGTGGACTTCCTGCCC
ACCACAGCCCAGCCAACCAAGAAATCCACCCTCAAGAAGCGCGTGTGCCGACTG
CCCCGCCCTGAAACCCAGAAGGGCCCTCTGTGCTCCCCCATCACCCTTGGACTGC
TGGTGGCGGGAGTCCTGGTGCTGCTCGTATCTCTGGGTGTCGCCATCCACCTGTG
CTGCCGCCGCCGCCGCGCCCGCCTGAGGTTTATGAAACAGTTTTACAAGTGATAA
ATCGATGGAAGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGGCCCTG
GAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAAATTAAGTTGCATCA
TTTTGTCTGACTAGGTGTCCTTCTATAATATTATGGGGTGGAGGGGGGTGGTATG
GAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGGGGTCTATTGGGA
ACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATCTCCGCCTCCTGG
GTTCAAGCGATTCTCCTGCCTCAGCCTCCCGAGTTGTTGGGATTCCAGGCATGCA
TGACCAGGCTCAGCTAATTTTTGTTTTTTTGGTAGAGACGGGGTTTCACCATATTG
GCCAGGCTGGTCTCCAACTCCTAATCTCAGGTGATCTACCCACCTTGGCCTCCCA
AATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGTCCTTCTGATTACT
AGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAG
AAGTTGTGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGG
GTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGG
GAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTT
TGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTT
ACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTACGTGA
TTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGC
TTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGC
CGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGT
CTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGAT
AGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCG
CGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC
TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTG
CTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCT
GGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGC
TGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAGT

CACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTGACTC
CACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGT
ACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTG
AGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGA
ATTTGCCCTTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTC
AAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAACTAGTCCAGTGTGGTGGAATTCT
GCAGATATCACGGCTAGCGCCACCATGGGTCGGGGGCTGCTCAGGGGCCTGTGG
CCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGCACGATCCCACCGCACGTTC
AGAAGTCGGTGAATAACGACATGATAGTCACTGACAACAACGGTGCAGTCAAGT
TTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACCAGAA
ATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGAAGT
CTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGTTTG
CCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTCCA
AAGTGCATTATGAAGGAGAAGAAAAAGCCTGGTGAGACTTTCTTCATGTGTTCCT
GTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATAACACCAG
CAATCCTGACTTGTTGCTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGCCA
CCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGTGAACC
GGCAGCAGAAGGCTAGTGGTTCAGGCGCAACGAATTTCTCTTTGCTGAAGCAGG
CTGGGGATGTCGAAGAAAATCCGGGTCCAATGGTGGGCTCGCTCAACTGCATCG
TAGCAGTCTCCCAGAATATGGGCATCGGGAAGAACGGTGATTTCCCGTGGCCCCC
ACTTCGCAACGAGAGCCGTTATTTCCAAAGAATGACTACAACCTCCTCCGTGGAG
GGTAAGCAGAACCTGGTCATCATGGGGAAGAAGACCTGGTTCTCTATCCCTGAA
AAAAACCGCCCCCTGAAGGGCCGCATCAACCTGGTGCTGAGCAGGGAACTCAAG
GAGCCTCCTCAGGGCGCGCATTTTCTGAGCCGCTCATTGGATGACGCTCTCAAAC
TGACCGAACAGCCGGAGCTAGCCAACAAGGTGGACATGGTGTGGATCGTCGGAG
GCTCCTCCGTGTACAAGGAGGCCATGAATCACCCCGGCCACTTGAAGCTGTTCGT
CACCCGGATCATGCAGGACTTCGAGTCGGACACGTTCTTTCCAGAGATTGACCTG
GAGAAGTACAAGCTGCTGCCCGAGTACCCGGGAGTTCTTAGTGATGTGCAGGAG
GAGAAAGGCATCAAGTACAAATTTGAGGTGTACGAGAAGAACGACTAACGGTCC
GTCCTGACCAATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATA
ATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTC
ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGTA
TACAGGTTACCTCAGTCTCCTAGGTACGTCTTATATCTATGAAAAAACATTCAAA
AGCACAACATCTAGAAGAACTTACCTTTTTTCACCACTCTATTGCAAAGATATGT
ACCGATTTCTCTCGAAGTACAAAAAACCGCTAGTTTTCAAATTCACCTCAAGACT
TTGAAAAAAAATTGAATCTGTCAATGTCAAATAAAATCAGAAACAAATGTCATA
ATGTTACGTTAATGTTGTCAGGTCGAAAAATAAAATTGCAAATAGAAATTTTGTT
CCTTTTTTATTGGTTTTTATTGGTGGGAAAAATATTCCCTCTAACTGCAAAAGGGT
TAATTATGTTAGAGGTAGAGTCGAC

* For certain depicted vectors, MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

Table 4

MAGEC2 TCR 8-3 MGTM codon optimized sequence (also known as "Leiden-8-3")

Alpha chain:
TRAV24*01 F/TRAJ32*02/MGTM modified TRAC

Alpha chain DNA sequence
ATGGAGAAGAATCCTTTGGCAGCCCCACTTCTTATCCTCTGGTTTCATCTTGACTG
CGTGAGCAGCATTCTGAACGTGGAACAAAGTCCTCAGTCACTGCATGTTCAGGA
GGGAGACAGCACCAATTTCACCTGCAGCTTCCCT**TCCAGCAATTTTTATGCC**CT
TCACTGGTACAGATGGGAAACTGCAAAAAGCCCCGAGGCCTTGTTTGTT**ATGAC**
TCTTAATGGGGATGAAAAGAAGAAAGGACGCATTAGTGCCACTCTTAATACCA
AGGAGGGTTACAGCTATTTGTATATCAAAGGATCCCAGCCTGAGGACTCAGCCA
CATACCTCTGT**GCCTCCGGAAGTGGTGGTGCTACAAACAAGCTCATC**TTTGGA
ACTGGCACTCTGCTTGCTGTCCAGCCAAacatccagaaccccgaccccgccgtgtaccagctgagggact
ccaagtccagcgacaagagcgtgtgtctgtttacggacttcgacagccagaccaacgtgagtcaaagcaaggacagcgacgtctaca
taacggataagaccgtgctggacatgcggagcatggacttcaagagcaacagcgccgtggcctggtccaacaagagcgacttcgcc
tgcgccaacgccttcaacaacagcatcatccccgaggacaccttcttccccagcagcgacgtgccctgcgacgtgaaactggtggag
aagtccttcgagacagacaccaatctgaacttcagaacctgctggtgatcgtgctgcggattctgctgctgaaagtggccggcttcaat
ctgctgatgaccctgcggctgtggagcagc (SEQ ID NO: 32)

Alpha chain protein sequence
MEKNPLAAPLLILWFHLDCVSSILNVEQSPQSLHVQEGDSTNFTCSFP**SSNFYA**LHWY
RWETAKSPEALFV**MTLNGDE**KKKGRISATLNTKEGYSYLYIKGSQPEDSATYLC**AS**
GSGGATNKLIFGTGTLLAVQPNiqnpdpavyqlrdskssdksvclftdfdsqtnvsqskdsdvyitdktvldmr
smdfksnsavawsnksdfacanafnnsiipedtffpssdvpcdvklveksfetdtnlnfqnllvivlrilllkvagfnllmtlrlwss
(SEQ ID NO: 33)

Beta chain:
TRBV16*01/TRBJ1-1*01/ MGTM modified TRBC

Beta chain DNA sequence
ATGAGCCCAATTTTCACCTGCATCACAATCCTTTGTCTGCTGGCTGCAGGTTCTCC
TGGTGAAGAAGTCGCCCAGACTCCAAAACATCTTGTCAGAGGGGAAGGACAGAA
AGCAAAACTTTATTGTGCCCCAATT**AAAGGACACAGTTAT**GTTTTCTGGTACCAA
CAGGTCCTGAAAAACGAGTTCAAGTTCTTGATTTCC**TTCCAGAATGAAAATGTC**
TTTGATGAAACAGGTATGCCCAAGGAAAGATTTTCAGCTAAGTGCCTCCCAAATT
CACCCTGTAGCCTTGAGATCCAGGCTACTAAGCTTGAGGATTCAGCAGTGTATTT
TTGT**GCCAGCAGCCAATCACGGAGCCTTAGGGGCACTGAAGCTTTC**TTTGGA
CAAGGCACCAGACTCACAGTTGTTGaagatctgaacaaggtgttcccctccagaggtggccgtgttcgagcctt
ctaaggccgagatcgcccacacacaaaaagccacccctcgtgtgcctggccaccggcttttttccccgaccacgtggaactgtcttggtg
ggtcaacggcaaagaggtgcactccggcgtgtcaacggatccccagcctctgaaagaacagcctgccctgaacgacagccggtact
gcctgagctccagactgagagtgtccgccaccttctggcagaaccccccggaaccacttcagatgccaggtgcagttttacggcctgag
cgagaacgacgagtggacccaggacagagccaagccccgtgacacaaatcgtgtctgccgaagcctggggaagagccgattgcgg
catcaccagcgcctcctatcaccagggcgtgctgagcgccacaatcctgtacgaaatcctgctgggcaaggccaccctgtacgccgt
gctggtgtctgctctggtgctgatggccatggtcaagcggaaggacttt

Beta chain protein sequence
MSPIFTCITILCLLAAGSPGEEVAQTPKHLVRGEGQKAKLYCAPI**KGHSY**VFWYQQV
LKNEFKFLIS**FQNENV**FDETGMPKERFSAKCLPNSPCSLEIQATKLEDSAVYFC**ASSQ**
SRSLRGTEAFFGQGTRLTVVEdlnkvfppevavfepskaeiahtqkatlvclatgffpdhvelswwvngkevhs
gvstdpqplkeqpalndsryclssrlrvsatfwqnprnhfrcqvqfyglsendewtqdrakpvtqivsaeawgradcgitsasyhq
gvlsatilyeillgkatlyavlvsalvlmamvkrkdf

Complete Beta and Alpha ORF DNA Sequence

ATGAGCCCAATTTTCACCTGCATCACAATCCTTTGTCTGCTGGCTGCAGGTTCTCC
TGGTGAAGAAGTCGCCCAGACTCCAAAACATCTTGTCAGAGGGGAAGGACAGAA
AGCAAAACTTTATTGTGCCCCAATT**AAAGGACACAGTTAT**GTTTTCTGGTACCA
ACAGGTCCTGAAAAACGAGTTCAAGTTCTTGATTTCC**TTCCAGAATGAAAATGT**
CTTTGATGAAACAGGTATGCCCAAGGAAAGATTTTCAGCTAAGTGCCTCCCAAAT
TCACCCTGTAGCCTTGAGATCCAGGCTACTAAGCTTGAGGATTCAGCAGTGTATT
TTTGT**GCCAGCAGCCAATCACGGAGCCTTAGGGGCACTGAAGCTTTC**TTTGG
ACAAGGCACCAGACTCACAGTTGTTGaagatctgaacaaggtgttcctccagaggtggccgtgttcgagcc
ttctaaggccgagatcgcccacacacaaaaagccacctctgtgtgcctggccaccggcttttcccccgaccacgtggaactgtcttggt
gggtcaacggcaaagaggtgcactccggcgtgtcaacggatccccagcctctgaaagaacagcctgccctgaacgacagccggta
ctgcctgagctccagactgagagtgtccgccaccttctggcagaaccccccggaaccacttcagatgccaggtgcagttttacggcctg
agcgagaacgacgagtggacccaggacagagccaagcccgtgacacaaatcgtgtctgccgaagcctggggaagagccgattgc
ggcatcaccagcgcctcctatcaccagggcgtgctgagcgccacaatcctgtacgaaatcctgctgggcaaggccaccctgtacgcc
gtgctggtgtctgctctggtgctgatggccatggtcaagcggaaggactttggcagcggcagagccaaaaggtccgggagcggtg
cgacaaacttagcctgttgaaacaagccggcgacgttgaagagaaccccggacctATGGAGAAGAATCCTTTG
GCAGCCCCACTTCTTATCCTCTGGTTTCATCTTGACTGCGTGAGCAGCATTCTGAA
CGTGGAACAAAGTCCTCAGTCACTGCATGTTCAGGAGGGAGACAGCACCAATTT
CACCTGCAGCTTCCCT**TCCAGCAATTTTTATGCC**CTTCACTGGTACAGATGGGA
AACTGCAAAAAGCCCCGAGGCCTTGTTTGTT**ATGACTCTTAATGGGGATGAA**A
AGAAGAAAGGACGCATTAGTGCCACTCTTAATACCAAGGAGGGTTACAGCTATT
TGTATATCAAAGGATCCCAGCCTGAGGACTCAGCCACATACCTCTGT**GCCTCCG**
GAAGTGGTGGTGCTACAAACAAGCTCATCTTTGGAACTGGCACTCTGCTTGCT
GTCCAGCCAAacatccagaaccccgaccccgccgtgtaccagctgagggactccaagtccagcgacaagagcgtgtgtct
gtttacggacttcgacagccagaccaacgtgagtcaaagcaaggacagcgacgtctacataacggataagaccgtgctggacatgc
ggagcatggacttcaagagcaacagcgccgtggcctggtccaacaagagcgacttcgcctgcgccaacgccttcaacaacagcatc
atccccgaggacaccttcttccccagcagcgacgtgccctgcgacgtgaaactggtggagaagtccttcgagacagacaccaatctg
aactttcagaacctgctggtgatcgtgctgcggattctgctgctgaaagtggccggcttcaatctgctgatgaccctgcggctgtggagc
agc

Complete Beta and Alpha ORF Protein Sequence

MSPIFTCITILCLLAAGSPGEEVAQTPKHLVRGEGQKAKLYCAPI**KGHSY**VFWYQQV
LKNEFKFLIS**FQNENV**FDETGMPKERFSAKCLPNSPCSLEIQATKLEDSAVYFC**ASSQ**
SRSLRGTEAFFGQGTRLTVVEdlnkvfppevavfepskaeiahtqkatlvclatgffpdhvelswwvngkevhs
gvstdpqplkeqpalndsryclssrlrvsatfwqnprnhfrcqvqfyglsendewtqdrakpvtqivsaeawgradcgitsasyhq
gvlsatilyeillgkatlyavlvsalvlmamvkrkdf*gsgrakrsgsgatnfsllkqagdveenpgp*MEKNPLAAPLLIL
WFHLDCVSSILNVEQSPQSLHVQEGDSTNFTCSFP**SSNFYA**LHWYRWETAKSPEALF
V**MTLNGDE**KKKGRISATLNTKEGYSYLYIKGSQPEDSATYLC**ASGSGGATNKLI**FG
TGTLLAVQPNiqnpdpavyqlrdskssdksvclftdfdsqtnvsqskdsdvyitdktvldmrsmdfksnsavawsnksd
facanafnnsiipedtffpssdvpcdvklveksfetdtnlnfqnllvivlrilllkvagfnllmtlrlwss

Representative vector pNVVD186 _TCR-Leiden-8-3_MSCV-TCR-Leiden-8-3-CD8-EF1α-dnTGFbRII-DHFR

GCTAGCTGGCTTGTTGTCCACAACCATTAAACCTTAAAAGCTTTAAAAGCCTTAT
ATATTCTTTTTTTTCTTATAAAACTTAAAACCTTAGAGGCTATTTAAGTTGCTGAT
TTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACGTGAAACATGAGAGCT
TAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAGGGTTTAGTTCATTAA
ACATGAGAGCTTAGTACATTAAACATGAGAGCTTAGTACATACTATCAACAGGTT
GAACTGCTGATCTGTACAGTAGAATTGGTAAAGAGAGTTGTGTAAAATATTGAGT

TCGCACATCTTGTTGTCTGATTATTGATTTTTGGCGAAACCATTTGATCATATGAC
AAGATGTGTATCTACCTTAACTTAATGATTTTGATAAAAATCATTAGGTACCAAT
TACATTGCTTGCAATTAACCCTTTAACGGTTATAAGGATCTAGATGAGATAGAAA
GATTTGGTTTTCGGATTTGTGTTACATAAGATGCCTAAAATAAAAATTGAGATTC
AATTTTTTTTTAAACTTTTTTTTTAATTGGTGGTAAGAATATTCCCTCTACCTGTTTGA
GAGTAATGAAATTGTAGTATGATTTTTCAACAAACTAAAAAAACAACATAAATCT
CACATAATAACTTTATTTCAATCACACAATTGAATACCAATAGGTTGACAGTACT
TACCAGCCTGCAGGTGAAAGACCCCACCTGTAGGTTTGGCAAGTTAGCTTAA
GTAACGCCATTTTGCAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTT
CAGATCAAGGTTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATA
TCTGTGGTAAGCAGTTCCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCC
CAGATGCGGTCCCGCCCTCAGCAGTTTCTAGCGAACCATCAGATGTTTCCAG
GGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCA
GTTTGCTTCTTGCTTCTGTTTGTGTGCTTCTGCTCCCTGAGCTCAATAAAAG
AGCCCACAACCCCTCACTTGGTGGGCCAGTCCTCTGATAGACTGTGTCCCCT
GGATACCCGTACGGTACCGCTAGCGCCACC*ATGAGCCCAATTTTCACCTGCATCA*
CAATCCTTTGTCTGCTGGCTGCAGGTTCTCCTGGTGAAGAAGTCGCCCAGACTCCA
AAACATCTTGTCAGAGGGGAAGGACAGAAAGCAAAACTTTATTGTGCCCCAATTAA
AGGACACAGTTATGTTTTCTGGTACCAACAGGTCCTGAAAAACGAGTTCAAGTTCT
TGATTTCCTTCCAGAATGAAAATGTCTTTGATGAAACAGGTATGCCCAAGGAAAGA
TTTTCAGCTAAGTGCCTCCCAAATTCACCCTGTAGCCTTGAGATCCAGGCTACTAA
GCTTGAGGATTCAGCAGTGTATTTTTGTGCCAGCAGCCAATCACGGAGCCTTAGGG
GCACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTTGAAGATCTGAAC
AAGGTGTTCCCTCCAGAGGTGGCCGTGTTCGAGCCTTCTAAGGCCGAGATCGCCCA
CACACAAAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTCCCCGACCACGTGG
AACTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACTCCGGCGTGTCAACGGATCC
CCAGCCTCTGAAAGAACAGCCTGCCCTGAACGACAGCCGGTACTGCCTGAGCTCCA
GACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGGAACCACTTCAGATGCCAG
GTGCAGTTTTACGGCCTGAGCGAGAACGACGAGTGGACCCAGGACAGAGCCAAGC
CCGTGACACAAATCGTGTCTGCCGAAGCCTGGGGAAGAGCCGATTGCGGCATCAC
CAGCGCCTCCTATCACCAGGGCGTGCTGAGCGCCACAATCCTGTACGAAATCCTGC
TGGGCAAGGCCACCCTGTACGCCGTGCTGGTGTCTGCTCTGGTGCTGATGGCCAT
*GGTCAAGCGGAAGGACTTT*GGCAGCGGCAGAGCCAAAAGGTCCGGGAGCGGTGC
GACAAACTTTAGCCTGTTGAAACAAGCCGGCGACGTTGAAGAGAACCCCGGACC
TATGGAGAAGAATCCTTTGGCAGCCCCACTTCTTATCCTCTGGTTTCATCTT
GACTGCGTGAGCAGCATTCTGAACGTGGAACAAAGTCCTCAGTCACTGCAT
GTTCAGGAGGGAGACAGCACCAATTTCACCTGCAGCTTCCCTTCCAGCAATT
TTTATGCCCTTCACTGGTACAGATGGGAAACTGCAAAAAGCCCCGAGGCCTT
GTTTGTTATGACTCTTAATGGGGATGAAAAGAAGAAAGGACGCATTAGTGCC
ACTCTTAATACCAAGGAGGGTTACAGCTATTTGTATATCAAAGGATCCCAGC
CTGAGGACTCAGCCACATACCTCTGTGCCTCCGGAAGTGGTGGTGCTACAA
ACAAGCTCATCTTTGGAACTGGCACTCTGCTTGCTGTCCAGCCAAACATCCA
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GAGCGTGTGTCTGTTTACGGACTTCGACAGCCAGACCAACGTGAGTCAAAG
CAAGGACAGCGACGTCTACATAACGGATAAGACCGTGCTGGACATGCGGAG
CATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTT
CGCCTGCGCCAACGCCTTCAACAACAGCATCATCCCCGAGGACACCTTCTTC
CCCAGCAGCGACGTGCCCTGCGACGTGAAACTGGTGGAGAAGTCCTTCGAG
ACAGACACCAATCTGAACTTTCAGAACCTGCTGGTGATCGTGCTGCGGATTC
TGCTGCTGAAAGTGGCCGGCTTCAATCTGCTGATGACCCTGCGGCTGTGGA

GCAGCAGGGCTAAGAGGTCCGGCAGCGGAGCCACCAATTTTTCCCTGCTGAAAC
AGGCTGGTGACGTGGAAGAAAACCCTGGCCCCATGGCGCTGCCCGTCACCGCGCT
GCTGCTGCCCCTGGCGCTGCTGTTACACGCCGCTCGGCCAGAGCTTCCCACCCAGGG
CACATTCTCCAACGTGTCCACCAATGTGTCGGGAGGCGGCGGATCGTCCCAGTTCAGA
GTGTCCCCTCTGGACCGCACCTGGAACCTGGGCGAGACCGTGGAGCTGAAATGTCAG
GTCCTGCTGAGCAACCCGACCTCCGGGTGCAGTTGGCTGTTCCAGCCGCGTGGTGCT
GCCGCAAGCCCTACGTTCCTGCTTTACCTGAGCCAGAACAAGCCCAAGGCGGCCGAG
GGCCTGGACACCCAGAGATTCTCCGGCAAGCGCCTGGGGGACACATTCGTGCTTACT
TTGAGCGATTTCCGCAGAGAGAACGAGGGCTACTATTTCTGTTCGGCGCTGAGCAATT
CCATCATGTATTTCAGCCACTTTGTGCCAGTGTTCCTGCCTGCCAAGCCTACCACAACA
CCAGCTCCCCGTCCCCCGACTCCGGCGCCTACCATCGCGAGTCAACCGTTGAGCCTG
AGGCCTGAGGCTTGTCGGCCCGCTGCGGGGGGTGCCGTCCACACCAGGGGCCTCGA
CTTTGCGTGCGACATCTATATTTGGGCGCCTCTGGCGGGTACCTGCGGGGTGCTGCT
GCTGTCATTGGTGATTACCCTGTACTGCAATCACCGCAACCGCCGGCGGGTCTGTAAG
TGCCCACGGCCTGTGGTCAAGTCCGGTGACAAACCGTCGCTCTCGGCTCGCTACGTG
CGCGCTAAGCGCAGCGGTTCCGGGGCCACCAACTTTTCATTGCTGAAGCAGGCCG
GTGATGTGGAGGAGAATCCAGGGCCCATGCGCCCCAGGCTTTGGCTCCTTCTTGC
TGCTCAGCTCACTGTCTTGCATGGCAACTCCGTTCTGCAGCAGACTCCCGCCTAC
ATCAAGGTGCAGACGAACAAGATGGTGATGCTGTCATGCGAGGCCAAGATCTCT
CTTTCAAATATGAGAATTTATTGGCTACGACAGCGCCAGGCCCCCTCCAGCGACA
GCCACCACGAGTTCCTGGCGCTTTGGGATTCTGCTAAAGGCACCATCCATGGAGA
GGAGGTGGAACAGGAGAAGATAGCTGTCTTCCGCGACGCATCCCGCTTCATCCT
GAACCTGACCAGCGTGAAGCCGGAGGACAGCGGCATCTACTTCTGTATGATCGTT
GGCTCCCCCGAGCTGACCTTCGGCAAAGGCACCCAGCTGTCCGTGGTGGACTTCC
TGCCCACCACAGCCCAGCCAACCAAGAAATCCACCCTCAAGAAGCGCGTGTGCC
GACTGCCCCGCCCTGAAACCCAGAAGGGCCCTCTGTGCTCCCCCATCACCCTTGG
ACTGCTGGTGGCGGGAGTCCTGGTGCTGCTCGTATCTCTGGGTGTCGCCATCCAC
CTGTGCTGCCGCCGCCGCCGCGCCCGCCTGAGGTTTATGAAACAGTTTTACAAGT
GATAAATCGATGGAAGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCTCTCCTGG
CCCTGGAAGTTGCCACTCCAGTGCCCACCAGCCTTGTCCTAATAAAATTAAGTTG
CATCATTTTGTCTGACTAGGTGTCCTTCTATAATATTATGGGGTGGAGGGGGGTG
GTATGGAGCAAGGGGCAAGTTGGGAAGACAACCTGTAGGGCCTGCGGGGTCTAT
TGGGAACCAAGCTGGAGTGCAGTGGCACAATCTTGGCTCACTGCAATCTCCGCCT
CCTGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCCGAGTTGTTGGGATTCCAGGC
ATGCATGACCAGGCTCAGCTAATTTTTGTTTTTTTGGTAGAGACGGGGTTTCACC
ATATTGGCCAGGCTGGTCTCCAACTCCTAATCTCAGGTGATCTACCCACCTTGGC
CTCCCAAATTGCTGGGATTACAGGCGTGAACCACTGCTCCCTTCCCTGTCCTTCTG
ATTACTAGTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCC
CCGAGAAGTTGTGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGC
GCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGG
TGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAAC
GGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCC
TCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTGCAGTAC
GTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTT
GCGCTTAAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTG
GGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGAT
AAGTCTCTAGCCATTTAAAATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA
AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGG
GCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGG
GGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG

CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAA
GGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCC
CTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGT
GAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCATGTG
ACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTG
GAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCAC
ACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCT
TGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTG
GTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAACTAGTCCAGTGTGGTGGAA
TTCTGCAGATATCACGGCTAGCGCCACCATGGGTCGGGGGCTGCTCAGGGGCCTG
TGGCCGCTGCACATCGTCCTGTGGACGCGTATCGCCAGCACGATCCCACCGCACG
TTCAGAAGTCGGTGAATAACGACATGATAGTCACTGACAACAACGGTGCAGTCA
AGTTTCCACAACTGTGTAAATTTTGTGATGTGAGATTTTCCACCTGTGACAACCA
GAAATCCTGCATGAGCAACTGCAGCATCACCTCCATCTGTGAGAAGCCACAGGA
AGTCTGTGTGGCTGTATGGAGAAAGAATGACGAGAACATAACACTAGAGACAGT
TTGCCATGACCCCAAGCTCCCCTACCATGACTTTATTCTGGAAGATGCTGCTTCTC
CAAAGTGCATTATGAAGGAGAAGAAAAAGCCTGGTGAGACTTTCTTCATGTGTTC
CTGTAGCTCTGATGAGTGCAATGACAACATCATCTTCTCAGAAGAATATAACACC
AGCAATCCTGACTTGTTGCTAGTCATATTTCAAGTGACAGGCATCAGCCTCCTGC
CACCACTGGGAGTTGCCATATCTGTCATCATCATCTTCTACTGCTACCGCGTGAA
CCGGCAGCAGAAGGCTAGTGGTTCAGGCGCAACGAATTTCTCTTTGCTGAAGCA
GGCTGGGGATGTCGAAGAAAATCCGGGTCCAATGGTGGGCTCGCTCAACTGCAT
CGTAGCAGTCTCCCAGAATATGGGCATCGGGAAGAACGGTGATTTCCCGTGGCC
CCCACTTCGCAACGAGAGCCGTTATTTCCAAAGAATGACTACAACCTCCTCCGTG
GAGGGTAAGCAGAACCTGGTCATCATGGGGAAGAAGACCTGGTTCTCTATCCCT
GAAAAAAACCGCCCCCTGAAGGGCCGCATCAACCTGGTGCTGAGCAGGGAACTC
AAGGAGCCTCCTCAGGGCGCGCATTTTCTGAGCCGCTCATTGGATGACGCTCTCA
AACTGACCGAACAGCCGGAGCTAGCCAACAAGGTGGACATGGTGTGGATCGTCG
GAGGCTCCTCCGTGTACAAGGAGGCCATGAATCACCCCGGCCACTTGAAGCTGTT
CGTCACCCGGATCATGCAGGACTTCGAGTCGGACACGTTCTTTCCAGAGATTGAC
CTGGAGAAGTACAAGCTGCTGCCCGAGTACCCGGGAGTTCTTAGTGATGTGCAG
GAGGAGAAAGGCATCAAGTACAAATTTGAGGTGTACGAGAAGAACGACTAACG
GTCCGTCCTGACCAATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCT
TATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTT
TTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTC
TGTATACAGGTTACCTCAGTCTCCTAGGTACGTCTTATATCTATGAAAAAACATT
CAAAAGCACAACATCTAGAAGAACTTACCTTTTTTCACCACTCTATTGCAAAGAT
ATGTACCGATTTCTCTCGAAGTACAAAAAACCGCTAGTTTTCAAATTCACCTCAA
GACTTTGAAAAAAAATTGAATCTGTCAATGTCAAATAAAATCAGAAACAAATGT
CATAATGTTACGTTAATGTTGTCAGGTCGAAAAATAAAATTGCAAATAGAAATTT
TGTTCCTTTTTTATTGGTTTTTATTGGTGGGAAAAATATTCCCTCTAACTGCAAAA
GGGTTAATTATGTTAGAGGTAGAGTCGAC

* For certain depicted vectors, MSCV promoter is in bold. Beta chain is annotated using bold and italic text. Alpha chain is annotated using bold and underlined text. CD34-enrichment tag (Q tag) is annotated using italic and underlined text. CD8-alpha is in italic. CD8-beta is underlined.

* Included in Tables 1-4 are peptide epitopes, as well as polypeptide molecules comprising an amino acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with an amino acid sequence of any sequences listed in Tables 1-3, or a portion thereof. Such polypeptides may have a function of the full-length peptide or polypeptide as described further herein.

* Included in Tables 1-4 are RNA nucleic acid molecules (*e.g.*, thymines replaced with uredines), nucleic acid molecules encoding orthologs of the encoded proteins, as well as DNA or RNA nucleic acid sequences comprising a nucleic acid sequence having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or more identity across their full length with the nucleic acid sequence of any sequence listed in Tables 1-43, or a portion thereof. Such nucleic acid molecules can have a function of the full-length nucleic acid as described further herein.

In some embodiments, the binding proteins provided herein comprise a constant region that is chimeric, humanized, human, primate, or rodent (*e.g.*, rat or mouse). For example, a human variable region may be chimerized with a murine constant region or a murine variable region may be humanized with a human constant region and/or human framework regions. In some embodiments, the constant regions may be mutated to modify functionality (*e.g.*, introduction of non-naturally occurring cysteine substitutions in opposing residue locations in TCR alpha and beta chains to provide disulfide bonds useful for increasing affinity between the TCR alpha and beta chains). Similarly, mutations may be made in the transmembrane domain of the constant region to modify functionality (*e.g.*, to increase hydrophobicity by introducing a non-naturally occurring substitution of a residue with a hydrophobic amino acid). In some embodiments, each CDR of the binding protein has up to five amino acid substitutions, insertions, deletions, or a combination thereof as compared to a reference CDR sequence. In some embodiments, mutations may be made to the constant region to increase cell surface expression.

In some embodiments, the binding proteins disclosed herein may be engineered protein scaffolds, an antibody or an antigen-binding fragment thereof, TCR-mimic antibodies, and the like. Such binding moieties may be designed and/or generated against peptides and/or MHC-peptide complexes described herein using routine immunological methods, such as immunizing a host, obtaining antibody-producing cells and/or antibodies

thereof, and generating hybridomas useful for producing monoclonal antibodies (*e.g.*, Watt *et al.* (2006) *Nat. Biotechnol.* 24:177-183; Gebauer and Skerra (2009) *Curr. Opin. Chem Biol.* 13:245-255; Skerra *et al.* (2008) *FEBS J.* 275:2677-2683; Nygren *et al.* (2008) *FEBS J.* 275:2668-2676; Dana *et al.* (2012) *Exp. Rev. Mol. Med.* 14:e6; Sergeva *et al.* (2011) *Blood* 117:4262-4272; PCT Publ. Nos. WO 2007/143104, PCT/US86/02269, and WO 86/01533; U.S. Pat. No. 4,816,567; Better *et al.* (1988) *Science* 240:1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. U.S.A.* 84:3439-3443; Liu *et al.* (1987) *J. Immunol.* 139:3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci.* 84:214-218; Nishimura *et al.* (1987) *Cancer Res.* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80:1553-1559); Morrison, S. L. (1985) *Science* 229:1202-1207; Oi *et al.* (1986) *Biotechniques* 4:214; U.S. Pat. No. 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J. Immunol.* 141:4053-4060. If desired, binding moieties may be isolated or purified using conventional procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, affinity chromatography, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography, lectin chromatography, and high performance liquid chromatography (HPLC) (*e.g.*, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y.).

The terms "antibody" and "antibodies" broadly encompass naturally-occurring forms of antibodies (*e.g.* IgG, IgA, IgM, IgE) and recombinant antibodies, such as single-chain antibodies, chimeric and humanized antibodies and multi-specific antibodies, as well as fragments and derivatives of all of the foregoing, which fragments and derivatives have at least an antigenic binding site. Antibody derivatives may comprise a protein or chemical moiety conjugated to an antibody.

In addition, intrabodies are well-known antigen-binding molecules having the characteristic of antibodies, but that are capable of being expressed within cells in order to bind and/or inhibit intracellular targets of interest (Chen *et al.* (1994) *Human Gene Ther.* 5:595-601). Methods are well-known in the art for adapting antibodies to target (*e.g.*, inhibit) intracellular moieties, such as the use of single-chain antibodies (scFvs), modification of immunoglobulin VL domains for hyperstability, modification of antibodies to resist the reducing intracellular environment, generating fusion proteins that increase intracellular stability and/or modulate intracellular localization, and the like. Intracellular antibodies can

also be introduced and expressed in one or more cells, tissues or organs of a multicellular organism, for example for prophylactic and/or therapeutic purposes (*e.g.*, as a gene therapy) (see, at least PCT Publ. Nos. WO 08/020079, WO 94/02610, WO 95/22618, and WO 03/014960; U.S. Pat. No. 7,004,940; Cattaneo and Biocca (1997) *Intracellular Antibodies: Development and Applications* (Landes and Springer-Verlag publs.); Kontermann (2004) *Methods* 34:163-170; Cohen *et al.* (1998) *Oncogene* 17:2445-2456; Auf der Maur *et al.* (2001) *FEBS Lett.* 508:407-412; Shaki-Loewenstein *et al.* (2005) *J. Immunol. Meth.* 303:19-39).

The term "antibody" as used herein also includes an "antigen-binding portion" of an antibody (or simply "antibody portion"). The term "antigen-binding portion", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically and/or selectively bind to an antigen (*e.g.*, a peptide and/or an MHC-peptide complex described herein). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent polypeptides (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Osbourn *et al.* 1998, Nature Biotechnology 16: 778). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Any VH and VL sequences of specific scFv can be linked to human immunoglobulin constant region cDNA or genomic sequences, in order to generate expression vectors encoding complete IgG polypeptides or other isotypes. VH and VL can also be used in the generation of Fab, Fv or other fragments of immunoglobulins using either protein chemistry or recombinant DNA technology. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in

which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-6448; Poljak *et al.* (1994) *Structure* 2:1121-1123).

Still further, an antibody or antigen-binding portion thereof may be part of larger immunoadhesion polypeptides, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion polypeptides include use of the streptavidin core region to make a tetrameric scFv polypeptide (Kipriyanov *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, protein subunit peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv polypeptides (Kipriyanov *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion polypeptides can be obtained using standard recombinant DNA techniques, as described herein.

Antibodies may be polyclonal or monoclonal; xenogeneic, allogeneic, or syngeneic; or modified forms thereof (*e.g.* humanized, chimeric, etc.). Antibodies may also be fully human. Preferably, antibodies of the invention bind specifically and/or selectively or substantially specifically and/or selectively to a peptide and/or an MHC-peptide complex described herein. The terms "monoclonal antibodies" and "monoclonal antibody composition", as used herein, refer to a population of antibody polypeptides that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of an antigen, whereas the term "polyclonal antibodies" and "polyclonal antibody composition" refer to a population of antibody polypeptides that contain multiple species of antigen binding sites capable of interacting with a particular antigen. A monoclonal antibody composition typically displays a single binding affinity for a particular antigen with which it immunoreacts.

Similar to other binding moieties described herein, antibodies may also be "humanized," which is intended to include antibodies made by a non-human cell having variable and constant regions which have been altered to more closely resemble antibodies that would be made by a human cell. For example, by altering the non-human antibody

amino acid sequence to incorporate amino acids found in human germline immunoglobulin sequences. The humanized antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro*/*ex vivo* or by somatic mutation *in vivo*), for example in the CDRs. The term "humanized antibody", as used herein, also includes antibodies in which CDR sequences derived from the germline of another mammalian species, have been grafted onto human framework sequences.

In some embodiments, the binding proteins disclosed herein may comprise a T cell receptor (TCR), an antigen-binding fragment of a TCR, or a chimeric antigen receptor (CAR). In some embodiments, the binding protein disclosed herein may comprise two polypeptide chains, each of which comprises a variable region comprising a CDR3 of a TCR alpha chain and a CDR3 of a TCR beta chain, or a CDR1, CDR2, and CDR3 of both a TCR alpha chain and a TCR beta chain. In some embodiments, a binding protein comprises a single chain TCR (scTCR), which comprises both the TCR V_α and TCR V_β domains, but only a single TCR constant domain (C_α or C_β). The term "chimeric antigen receptor" (CAR) refers to a fusion protein that is engineered to contain two or more naturally-occurring amino acid sequences linked together in a way that does not occur naturally or does not occur naturally in a host cell, which fusion protein can function as a receptor when present on a surface of a cell. CARs encompassed by the present invention may include an extracellular portion comprising an antigen-binding domain (*i.e.*, obtained or derived from an immunoglobulin or immunoglobulin-like molecule, such as an antibody or TCR, or an antigen binding domain derived or obtained from a killer immunoreceptor from an NK cell) linked to a transmembrane domain and one or more intracellular signaling domains (optionally containing co-stimulatory domain(s)) (see, *e.g.*, Sadelain *et al.* (2013) *Cancer Discov.* 3:388, Harris and Kranz (2016) *Trends Pharmacol. Sci.* 37:220, and Stone *et al.* (2014) *Cancer Immunol. Immunother.* 63:1163).

In some embodiments, 1) the TCR alpha chain CDR, TCR V_α domain, and/or TCR alpha chain is encoded by a TRAV, TRAJ, and/or TRAC gene or fragment thereof selected from the group of TRAV, TRAJ, and TRAC genes listed in Table 2, and/or 2) the TCR beta chain CDR, TCR V_β domain, and/or TCR beta chain is encoded by a TRBV, TRBJ, and/or TRBC gene or fragment thereof selected from the group of TRBV, TRBJ, and TRBC genes listed in Table 2, and/or 3) each CDR of the binding protein has up to five amino acid

substitutions, insertions, deletions, or a combination thereof as compared to the cognate reference CDR sequence listed in Table 2.

In some embodiments, the binding proteins (*e.g.*, the TCR, antigen-binding fragment of a TCR, or chimeric antigen receptor (CAR)) disclosed herein 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.

Methods for producing engineered binding proteins, such as TCRs, CARs, and antigen-binding fragments thereof, are well-known in the art (*e.g.*, Bowerman *et al.* (2009) *Mol. Immunol.*. 5:3000; U.S. Pat. No. 6,410,319; U.S. Pat. No. 7,446,191; U.S. Pat. Publ. No. 2010/065818; U.S. Pat. No. 8,822,647; PCT Publ. No. WO 2014/031687; U.S. Pat. No. 7,514,537; and Brentjens *et al.* (2007) *Clin..Cancer Res.* 73:5426).

In some embodiments, the binding protein described herein is a TCR, or antigen-binding fragment thereof, expressed on a cell surface, wherein the cell surface-expressed TCR is capable of more efficiently associating with a CD3 protein as compared to endogenous TCR. A binding protein encompassed by the present invention, such as a TCR, when expressed on the surface of a cell like a T cell, may also have higher surface expression on the cell as compared to an endogenous binding protein, such as an endogenous TCR. In some embodiments, provided herein is a CAR, wherein the binding domain of the CAR comprises an antigen-specific TCR binding domain (see, *e.g.*, Walseng *et al.* (2017) *Scientific Reports* 7:10713).

Also provided are modified binding proteins (*e.g.*, TCRs, antigen-binding fragments of TCRs, or CARs) that may be prepared according to well-known methods using a binding protein having one or more of the V_α and/or V_β sequences disclosed herein as starting material to engineer a modified binding protein that may have altered properties from the starting binding protein. A binding protein may be engineered by modifying one or more residues within one or both variable regions (*i.e.*, V_α and/or V_β), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, a binding protein may be engineered by modifying residues within the constant region(s).

Another type of variable region modification is to mutate amino acid residues within the V_α and/or V_β CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the binding protein of interest. Site-directed mutagenesis

or PCR-mediated mutagenesis may be performed to introduce the mutation(s) and the effect on protein binding, or other functional property of interest, may be evaluated in *in vitro*, *ex vivo*, or *in vivo* assays as described herein and provided in the Examples. In some embodiments, conservative modifications (as discussed above) may be introduced. The mutations may be amino acid substitutions, additions or deletions. In some embodiments, the mutations are substitutions. Moreover, typically no more than one, two, three, four or five residues within a CDR region are modified.

In some embodiments, binding proteins (*e.g.,* TCRs, antigen-binding fragments of TCRs, or CARs) described herein may possess one or more amino acid substitutions, deletions, or additions relative to a naturally occurring TCR. In some embodiments, each CDR of the binding protein has up to five amino acid substitutions, insertions, deletions, or a combination thereof as compared to the cognate reference CDR sequence listed in Table 2. Conservative substitutions of amino acids are well-known and may occur naturally or may be introduced when the binding protein is recombinantly produced. Amino acid substitutions, deletions, and additions may be introduced into a protein using mutagenesis methods known in the art (see, *e.g.,* Sambrook *et al.* (2001) *Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Laboratory Press, NY). Oligonucleotide-directed site-specific (or segment specific) mutagenesis procedures may be employed to provide an altered polynucleotide that has particular codons altered according to the substitution, deletion, or insertion desired. Alternatively, random or saturation mutagenesis techniques, such as alanine scanning mutagenesis, error prone polymerase chain reaction mutagenesis, and oligonucleotide-directed mutagenesis may be used to prepare immunogen polypeptide variants (see, *e.g.,* Sambrook *et al.* supra).

A variety of criteria known to the ordinarily skilled artisan 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 some embodiments, 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).

In some embodiments, an encoded binding protein (*e.g.*, TCR, antigen-binding fragment of a TCR, or CAR) may comprise a "signal peptide" (also known as a leader sequence, leader peptide, or transit peptide). Signal peptides target newly synthesized polypeptides to their appropriate location inside or outside the cell. A signal peptide may be removed from the polypeptide during or once localization or secretion is completed. Polypeptides that have a signal peptide are referred to herein as a "pre-protein" and polypeptides having their signal peptide removed are referred to herein as "mature" proteins or polypeptides. In some embodiments, a binding protein (*e.g.*, TCR, antigen-binding fragment of a TCR, or CAR) described herein comprises a mature V_α domain, a mature V_β domain, or both. In some embodiments, a binding protein (*e.g.*, TCR, antigen-binding fragment of a TCR, or CAR) described herein comprises a mature TCR β-chain, a mature TCR α-chain, or both.

In some embodiments, the binding proteins are fusion proteins comprising: (a) an extracellular component comprising a TCR or antigen-binding fragment thereof; (b) an intracellular component comprising an effector domain or a functional portion thereof; and (c) a transmembrane domain connecting the extracellular and intracellular components. In some embodiments, the fusion protein is capable of binding (*e.g.*, specifically and/or selectively) to a peptide-MHC (pMHC) complex comprising a MAGEC2 immunogenic peptide in the context of an MHC molecule (*e.g.*, an MHC class I molecule). In some embodiments, the MHC molecule comprises an MHC alpha chain that is an HLA serotype HLA-B*07. In some embodiments, the HLA allele is selected from the group consisting of HLA-B*07:02.

As used herein, an "effector domain" or "immune effector domain" is an intracellular portion or domain of a fusion protein or receptor that can directly or indirectly promote an

immune response in a cell when receiving an appropriate signal. In some embodiments, an effector domain is from an immune cell protein or portion thereof or immune cell protein complex that receives a signal when bound (*e.g.*, CD3ζ), or when the immune cell protein or portion thereof or immune cell protein complex binds directly to a target molecule and triggers signal transduction from the effector domain in an immune cell.

An effector domain may directly promote a cellular response when it contains one or more signaling domains or motifs, such as an intracellular tyrosine-based activation motif (ITAM), such as those found in costimulatory molecules. Without wishing to be bound by theory, it is believed that ITAMs are useful for T cell activation following ligand engagement by a T cell receptor or by a fusion protein comprising a T cell effector domain. In some embodiments, the intracellular component or functional portion thereof comprises an ITAM. Exemplary immune effector domains include but are not limited to those from, CD3ε, CD3δ, CD3ζ, CD25, CD79A, CD79B, CARD11, DAP10, FcRα, FcRβ, FcRγ, Fyn, HVEM, ICOS, Lck, LAG3, LAT, LRP, NKG2D, NOTCH1, NOTCH2, NOTCH3, NOTCH4, Wnt, ROR2, Ryk, SLAMF1, Slp76, pTα, TCRα, TCRβ, TRIM, Zap70, PTCH2, or any combination thereof. In some embodiments, an effector domain comprises a lymphocyte receptor signaling domain (*e.g.*, CD3ζ or a functional portion or variant thereof).

In further embodiments, the intracellular component of the fusion protein comprises a costimulatory domain or a functional portion thereof selected from CD27, CD28, 4-1BB (CD137), OX40 (CD134), CD2, CD5, ICAM-l (CD54), LFA-l (CD11a/CD18), ICOS (CD278), GITR, CD30, CD40, BAFF-R, HVEM, LIGHT, MKG2C, SLAMF7, NKp80, CD160, B7-H3, a ligand that binds (*e.g.*, specifically and/or selectively) with CD83, or a functional variant thereof, or any combination thereof. In some embodiments, the intracellular component comprises a CD28 costimulatory domain or a functional portion or variant thereof (which may optionally include a LL-GG mutation at positions 186-187 of the native CD28 protein (*e.g.*, Nguyen *et al.* (2003) *Blood* 702:4320), a 4-1BB costimulatory domain or a functional portion or variant thereof, or both.

In some embodiments, an effector domain comprises a CD3ε endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises a CD27 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises a CD28 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In still further embodiments, an effector domain

comprises a 4-1BB endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises an OX40 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises a CD2 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises a CD5 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises an ICAM-1 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises a LFA-1 endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof. In further embodiments, an effector domain comprises an ICOS endodomain or a functional (*e.g.*, signaling) portion thereof, or a functional variant thereof.

An extracellular component and an intracellular component encompassed by the present invention are connected by a transmembrane domain. A "transmembrane domain," as used herein, is a portion of a transmembrane protein that can insert into or span a cell membrane. Transmembrane domains have a three-dimensional structure that is thermodynamically stable in a cell membrane and generally range in length from about 15 amino acids to about 30 amino acids. The structure of a transmembrane domain may comprise an alpha helix, a beta barrel, a beta sheet, a beta helix, or any combination thereof. In some embodiments, the transmembrane domain comprises or is derived from a known transmembrane protein (*e.g.*, a CD4 transmembrane domain, a CD8 transmembrane domain, a CD27 transmembrane domain, a CD28 transmembrane domain, or any combination thereof).

In some embodiments, the extracellular component of the fusion protein further comprises a linker disposed between the binding domain and the transmembrane domain. As used herein when referring to a component of a fusion protein that connects the binding and transmembrane domains, a "linker" may be an amino acid sequence having from about two amino acids to about 500 amino acids, which can provide flexibility and room for conformational movement between two regions, domains, motifs, fragments, or modules connected by the linker. For example, a linker encompassed by the present invention can position the binding domain away from the surface of a host cell expressing the fusion protein to enable proper contact between the host cell and a target cell, antigen binding, and activation (Patel *et al.* (1999) *Gene Therapy* 6:412-419). Linker length may be varied to maximize antigen recognition based on the selected target molecule, selected binding epitope,

or antigen binding domain seize and affinity (see, *e.g.*, Guest *et al.* (2005) *Immunother.* 28:203-11 and PCT Publ. No. WO 2014/031687). Exemplary linkers include those having a glycine-serine amino acid chain having from one to about ten repeats of Gly_xSer_y, wherein x and y are each independently an integer from 0 to 10, provided that x and y are not both 0 (*e.g.*, $(Gly_4Ser)_2$, $(Gly_3Ser)_2$, Gly_2Ser, or a combination thereof, such as $((Gly_3Ser)_2Gly_2Ser)$).

A binding protein may be conjugated to an agent, such as a detection moiety, readiosensitizer, photosensitizer, and the like, and/or may be chemically modified as described above regarding peptides.

Binding proteins encompassed by the present invention may, in some embodiments, be covalently linked to a moiety. In some embodiments, the covalently linked moiety comprises an affinity tag or a label. The affinity tag may be selected from the group consisting of Glutathione-S-Transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, HaloTag, HA tag, Flag tag, His tag, biotin tag, and V5 tag. The label may be a fluorescent protein. In some embodiments, the covalently linked moiety is selected from the group consisting of an inflammatory agent, an anti-inflammatory agent, a cytokine, a toxin, a cytotoxic molecule, a radioactive isotope, or an antibody such as a single-chain Fv.

A binding protein may be conjugated to an agent used in imaging, research, therapeutics, theranostics, pharmaceuticals, chemotherapy, chelation therapy, targeted drug delivery, and radiotherapy. In some embodiments, a binding protein may be conjugated to or fused with detectable agents, such as a fluorophore, a near-infrared dye, a contrast agent, a nanoparticle, a metal-containing nanoparticle, a metal chelate, an X-ray contrast agent, a PET agent, a metal, a radioisotope, a dye, radionuclide chelator, or another suitable material that can be used in imaging. In some embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more detectable moieties may be linked to a binding protein. Non-limiting examples of radioisotopes include alpha emitters, beta emitters, positron emitters, and gamma emitters. In some embodiments, the metal or radioisotope is selected from the group consisting of actinium, americium, bismuth, cadmium, cesium, cobalt, europium, gadolinium, iridium, lead, lutetium, manganese, palladium, polonium, radium, ruthenium, samarium, strontium, technetium, thallium, and yttrium. In some embodiments, the metal is actinium, bismuth, lead, radium, strontium, samarium, or yttrium. In some embodiments, the radioisotope is actinium-225 or lead-212. In some embodiments, the near-infrared dyes are not easily quenched by biological tissues and fluids. In some embodiments, the fluorophore is a fluorescent agent emitting electromagnetic radiation at a wavelength between 650 nm and 4000 nm, such emissions

being used to detect such agent. Non-limiting examples of fluorescent dyes that may be used as a conjugating molecule include DyLight-680, DyLight-750, VivoTag-750, DyLight-800, IRDye-800, VivoTag-680, Cy5.5, ZQ800, or indocyanine green (ICG). In some embodiments, near infrared dyes often include cyanine dyes (*e.g.*, Cy7, Cy5.5, and Cy5). Additional, non-limiting examples of fluorescent dyes for use as a conjugating molecule in accordance with present invention include acradine orange or yellow, Alexa Fluors® (*e.g.*, Alexa Fluor® 790, 750, 700, 680, 660, and 647) and any derivative thereof, 7-actinomycin D, 8-anilinonaphthalene-1-sulfonic acid, ATTO® dye and any derivative thereof, auramine-rhodamine stain and any derivative thereof, bensantrhone, bimane, 9-10-bis(phenylethynyl)anthracene, 5,12-bis(phenylethynyl)naththacene, bisbenzimide, brainbow, calcein, carbodyfluorescein and any derivative thereof, 1-chloro-9,10-bis(phenylethynyl)anthracene and any derivative thereof, DAPI, DiOC6, DyLight® Fluors® and any derivative thereof, epicocconone, ethidium bromide, FlAsH-EDT2®, Fluo dye and any derivative thereof, FluoProbe® and any derivative thereof, fluorescein and any derivative thereof, Fura® and any derivative thereof, GelGreen® and any derivative thereof, GelRed® and any derivative thereof, fluorescent proteins and any derivative thereof, m isoform proteins and any derivative thereof such as for example mCherry, hetamethine dye and any derivative thereof, hoeschst stain, iminocoumarin, indian yellow, indo-1 and any derivative thereof, laurdan, lucifer yellow and any derivative thereof, luciferin and any derivative thereof, luciferase and any derivative thereof, mercocyanine and any derivative thereof, nile dyes and any derivative thereof, perylene, phloxine, phyco dye and any derivative thereof, propium iodide, pyranine, rhodamine and any derivative thereof, ribogreen, RoGFP, rubrene, stilbene and any derivative thereof, sulforhodamine and any derivative thereof, SYBR and any derivative thereof, synapto-pHluorin, tetraphenyl butadiene, tetrasodium tris, Texas Red, Titan Yellow, TSQ, umbelliferone, violanthrone, yellow fluorescent protein and YOYO-1. Other suitable fluorescent dyes include, but are not limited to, fluorescein and fluorescein dyes (*e.g.*, fluorescein isothiocyanine or FITC, naphthofluorescein, 4', 5'-dichloro-2',7'-dimethoxyfluorescein, 6-carboxyfluorescein or FAM, *etc.*), carbocyanine, merocyanine, styryl dyes, oxonol dyes, phycoerythrin, erythrosin, eosin, rhodamine dyes (*e.g.*, carboxytetramethyl-rhodamine or TAMRA, carboxyrhodamine 6G, carboxy-X-rhodamine (ROX), lissamine rhodamine B, rhodamine 6G, rhodamine Green, rhodamine Red, tetramethylrhodamine (TMR), *etc.*), coumarin and coumarin dyes (*e.g.*, methoxycoumarin, dialkylaminocoumarin, hydroxycoumarin, aminomethylcoumarin (AMCA), *etc.*), Oregon Green™ dyes (*e.g.*, Oregon Green™ 488, 500, 514., *etc.*), Texas Red®, Texas Red®-X,

SPECTRUM RED®, SPECTRUM GREEN®, cyanine dyes (*e.g.*, CY-3, Cy-5, CY-3.5, CY-5.5, *etc.*), Alexa Fluor® dyes (*e.g.*, Alexa Fluor® 350, 488, 532, 546, 568, 594, 633, 660, 680, *etc.*), BODIPY® dyes (*e.g.*, BODIPY® FL, R6G, TMR, TR, 530/550, 558/568, 564/570, 576/589, 581/591, 630/650, 650/665, *etc.*), IRD dyes (*e.g.*, IRD40™, IRD700™, IRD800™, *etc.*), and the like. Additional suitable detectable agents are well-known in the art (*e.g.*, PCT Publ. No. PCT/US14/56177). Non-limiting examples of radioisotopes include alpha emitters, beta emitters, positron emitters, and gamma emitters. In some embodiments, the metal or radioisotope is selected from the group consisting of actinium, americium, bismuth, cadmium, cesium, cobalt, europium, gadolinium, iridium, lead, lutetium, manganese, palladium, polonium, radium, ruthenium, samarium, strontium, technetium, thallium, and yttrium. In some embodiments, the metal is actinium, bismuth, lead, radium, strontium, samarium, or yttrium. In some embodiments, the radioisotope is actinium-225 or lead-212.

Binding proteins may be conjugated to a radiosensitizer or photosensitizer. Examples of radiosensitizers include but are not limited to: ABT-263, ABT-199, WEHI-539, paclitaxel, carboplatin, cisplatin, oxaliplatin, gemcitabine, etanidazole, misonidazole, tirapazamine, and nucleic acid base derivatives (*e.g.*, halogenated purines or pyrimidines, such as 5-fluorodeoxyuridine). Examples of photosensitizers include but are not limited to: fluorescent molecules or beads that generate heat when illuminated, nanoparticles, porphyrins and porphyrin derivatives (*e.g.*, chlorins, bacteriochlorins, isobacteriochlorins, phthalocyanines, and naphthalocyanines), metalloporphyrins, metallophthalocyanines, angelicins, chalcogenapyrrillium dyes, chlorophylls, coumarins, flavins and related compounds such as alloxazine and riboflavin, fullerenes, pheophorbides, pyropheophorbides, cyanines (*e.g.*, merocyanine 540), pheophytins, sapphyrins, texaphyrins, purpurins, porphycenes, phenothiaziniums, methylene blue derivatives, naphthalimides, nile blue derivatives, quinones, perylenequinones (*e.g.*, hypericins, hypocrellins, and cercosporins), psoralens, quinones, retinoids, rhodamines, thiophenes, verdins, xanthene dyes (*e.g.*, eosins, erythrosins, rose bengals), dimeric and oligomeric forms of porphyrins, and prodrugs such as 5-aminolevulinic acid. Advantageously, this approach allows for highly specific targeting of cells of interest (*e.g.*, immune cells) using both a therapeutic agent (*e.g.*, drug) and electromagnetic energy (*e.g.*, radiation or light) concurrently. In some embodiments, the binding protein is fused with, or covalently or non-covalently linked to the agent, for example, directly or via a linker.

In some embodiments, the binding protein may be chemically modified. For example, a binding protein may be mutated to modify peptide properties such as detectability, stability, biodistribution, pharmacokinetics, half-life, surface charge, hydrophobicity, conjugation sites, pH, function, and the like. N-methylation is one example of methylation that can occur in a binding protein encompassed by the present invention. In some embodiments, a binding protein may be modified by methylation on free amines such as by reductive methylation with formaldehyde and sodium cyanoborohydride.

A chemical modification may comprise a polymer, a polyether, polyethylene glycol, a biopolymer, a zwitterionic polymer, a polyamino acid, a fatty acid, a dendrimer, an Fc region, a simple saturated carbon chain such as palmitate or myristolate, or albumin. The chemical modification of a binding protein with an Fc region may be a fusion Fc-protein. A polyamino acid may include, for example, a poly amino acid sequence with repeated single amino acids (*e.g.,* poly glycine), and a poly amino acid sequence with mixed poly amino acid sequences that may or may not follow a pattern, or any combination of the foregoing.

In some embodiments, the binding proteins encompassed by the present invention may be modified. In some embodiments, the modifications having substantial or significant sequence identity to a parent binding protein to generate a functional variant that maintains one or more biophysical and/or biological activities of the parent binding protein (*e.g.*, maintain pMHC binding specificity). In some embodiments, the mutation is a conservative amino acid substitution.

In some embodiments, binding proteins encompassed by the present invention may comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are well-known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, a-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1 ,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, a-aminocyclopentane carboxylic acid, oc-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic acid, a-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, ,β-diaminopropionic acid, homophenylalanine, and oc-tert-butylglycine.

Binding proteins encompassed by the present invention may be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized (*e.g.*, via a disulfide bridge), or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

In some embodiments, the attachment of a hydrophobic moiety, such as to the N-terminus, the C-terminus, or an internal amino acid, may be used to extend half-life of a peptide encompassed by the present invention. In other embodiments, a binding protein may include post-translational modifications (*e.g.*, methylation and/or amidation), which can affect, for example, serum half-life. In some embodiments, simple carbon chains (*e.g.*, by myristoylation and/or palmitylation) may be conjugated to the binding proteins. In some embodiments, the simple carbon chains may render the binding proteins easily separable from the unconjugated material. For example, methods that may be used to separate the binding proteins from the unconjugated material include, but are not limited to, solvent extraction and reverse phase chromatography. The lipophilic moieties can extend half-life through reversible binding to serum albumin. The conjugated moieties may be lipophilic moieties that extend half-life of the peptides through reversible binding to serum albumin. In some embodiments, the lipophilic moiety may be cholesterol or a cholesterol derivative, including cholestenes, cholestanes, cholestadienes and oxysterols. In some embodiments, the binding proteins may be conjugated to myristic acid (tetradecanoic acid) or a derivative thereof. In other embodiments, a binding protein may be coupled (*e.g.*, conjugated) to a half-life modifying agent. Examples of half-life modifying agents include but are not limited to: a polymer, a polyethylene glycol (PEG), a hydroxyethyl starch, polyvinyl alcohol, a water soluble polymer, a zwitterionic water soluble polymer, a water soluble poly(amino acid), a water soluble polymer of proline, alanine and serine, a water soluble polymer containing glycine, glutamic acid, and serine, an Fc region, a fatty acid, palmitic acid, or a molecule that binds to albumin. In some embodiments, a spacer or linker may be coupled to a binding protein, such as 1, 2, 3, 4, or more amino acid residues that serve as a spacer or linker in order to facilitate conjugation or fusion to another molecule, as well as to facilitate cleavage of the peptide from such conjugated or fused molecules. In some embodiments, binding proteins may be conjugated to other moieties that, for example, can modify or effect changes to the properties of the binding proteins.

A binding protein may be produced recombinantly or synthetically, such as by solid-phase peptide synthesis or solution-phase peptide synthesis. Polypeptide synthesis may be performed by known synthetic methods, such as using fluorenylmethyloxycarbonyl (Fmoc)

chemistry or by butyloxycarbonyl (Boc) chemistry. Polypeptide fragments may be joined together enzymatically or synthetically.

In an aspect encompassed by the present invention, provided herein are methods of producing a binding protein described herein, comprising the steps of: (i) culturing a transformed host cell which has been transformed by a nucleic acid comprising a sequence encoding a binding protein described herein under conditions suitable to allow expression of said binding protein; and (ii) recovering the expressed binding protein.

Methods useful for isolating and purifying recombinantly produced binding protein, by way of example, may include obtaining supernatants from suitable host cell/vector systems that secrete the binding protein 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 binding proteins described herein include batch cell culture, which is monitored and controlled to maintain appropriate culture conditions. Purification of the binding protein may be performed according to methods described herein and known in the art.

In any of the herein disclosed embodiments, the encoded binding protein is capable of bind to a peptide–MHC (pMHC) complex comprising a MAGEC2 immunogenic peptide in the context of an MHC molecule (*e.g.*, an MHC class I molecule). In some embodiments, the MHC molecule comprises an MHC alpha chain that is an HLA serotype HLA-B*07. In some embodiments, the HLA allele is HLA-B*07:02.

A variety of assays are well-known for assessing binding affinity and/or determining whether a binding molecule binds (*e.g.*, specifically and/or selectively) to a particular ligand (*e.g.*, peptide antigen-MHC complex). It is within the level of a skilled artisan to determine the binding affinity of a binding protein for a target, such as a T cell peptide epitope of a target polypeptide, such as by using any of a number of binding assays that are well-known in the art. For example, in some embodiments, a Biacore™ machine may be used to determine the binding constant of a complex between two proteins. The dissociation constant (K_D) for the complex may be determined by monitoring changes in the refractive index with respect to time as buffer is passed over the chip. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays such as enzyme linked

immunosorbent assays (ELISA) and radioimmunoas says (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance (NMR). Other exemplary assays include, but are not limited to, Western blot, ELISA, analytical ultracentrifugation, spectroscopy and surface plasmon resonance (Biacore™) analysis (see, *e.g.,* Scatchard *et al.* (1949) *Ann. N.Y. Acad. Sci.* 51:660, Wilson (2002) *Science* 295:2103, Wolff *et al.* (1993) *Cancer Res.* 53:2560, and U.S. Pat. Nos. 5,283,173 and 5,468,614), flow cytometry, sequencing and other methods for detection of expressed nucleic acids. In one example, apparent affinity for a target is measured by assessing binding to various concentrations of tetramers, for example, by flow cytometry using labeled multimers, such as MHC-antigen tetramers. In one representative example, apparent K_D of a binding protein 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.

VI. Nucleic Acids and Vectors

In an aspect encompassed by the present invention, provided herein are nucleic acid molecules that encode proteins described herein, such as MAGEC2 immunogenic peptides and fragments thereof, MHC molecules, binding proteins (*e.g.,* TCRs, antigen-binding fragments of the TCRs, CARs, and the like), and the like.

In some embodiments, the nucleic acid molecule hybridizes, under stringent conditions, with the complement of a sequence with at least about at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity, such as over the full length, to a nucleic acid encoding a polypeptide selected from the group consisting of the polypeptide sequences listed in Tables 1-3.

In some embodiments, the nucleic acid molecule hybridizes, under stringent conditions, with the complement of a nucleic acid encoding a polypeptide selected from the group consisting of polypeptide sequences listed in Tables 1-3.

In some embodiments, the nucleic acid molecule comprises (*e.g.,* comprises, consists essentially of, or consists of) a nucleotide sequence encoding a polypeptide selected from the group consisting of polypeptide sequences listed in Tables 1-3.

In some embodiments, the nucleic acid sequence encodes a MAGEC2 immunogenic peptides described herein.

In some embodiments, the nucleic acids comprise (*e.g.*, comprise, consist essentially of, or consist of) a nucleotide sequence encoding at least one (*e.g.*, one, two, or three) TCR α-chain CDR set forth in Table 2. In some embodiments, the nucleic acids comprise (*e.g.*, comprise, consist essentially of, or consist of) a nucleotide sequence encoding a TCR V_α domain having an amino acid sequence that is at least about at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR V_α domain sequence set forth in Table 2. In some embodiments, the nucleic acids comprise (*e.g.*, comprise, consist essentially of, or consist of) a nucleotide sequence encoding a TCR α-chain having an amino acid sequence that is at least about at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR α-chain sequence set forth in Table 2.

In some embodiments, the nucleic acids comprise (*e.g.*, comprise, consist essentially of, or consist of) a nucleotide sequence encoding at least one (*e.g.*, one, two, or three) TCR β-chain CDR set forth in Table 2. In some embodiments, the nucleic acids comprise (*e.g.*, comprise, consist essentially of, or consist of) a nucleotide sequence encoding a TCR V_β domain having an amino acid sequence that is at least about at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR V_β domain sequence set forth in Table 2. In some embodiments, the nucleic acids comprise (*e.g.*, comprise, consist essentially of, or consist of) a nucleotide sequence encoding a TCR β-chain having an amino acid sequence that is at least about at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identity to a TCR β-chain sequence set forth in Table 2.

The term "nucleic acid" includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which may be single-stranded or double-stranded, synthesized or obtained (*e.g.*, isolated and/or purified) from natural sources, which may contain natural, non-natural or altered nucleotides, and which may contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. In an embodiment, the nucleic acid comprises complementary DNA (cDNA).

In some embodiments, the nucleic acids encompassed by the present invention are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that may replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication may be *in vitro*, *ex vivo*, or *in vivo* replication.

The nucleic acids can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. See, for example, Green and Sambrook *et al.* supra. For example, a nucleic acid may be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed upon hybridization (*e.g.*, phosphorothioate derivatives and acridine substituted nucleotides). Examples of modified nucleotides that may be used to generate the nucleic acids include, but are not limited to, 5-fiuorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N^6-isopentenyladenine, 1 -methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N^6-substituted adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N^6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, and 2,6-diaminopurine. Alternatively, one or more of the nucleic acids encompassed by the present invention can be purchased from companies, such as Integrated DNA Technologies (Coralville, IA).

In one embodiment, the nucleic acid comprises a codon-optimized nucleotide sequence. Without being bound to a particular theory or mechanism, it is believed that codon optimization of the nucleotide sequence increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleotide sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency. In some

embodiments, the nucleotide sequences described herein are codon-optimized for expression in a host cell (*e.g.*, an immune cell, such as a T cell).

The present invention also provides a nucleic acid comprising a nucleotide sequence which is complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

The nucleotide sequence which hybridizes under stringent conditions may hybridize under high stringency conditions. By "high stringency conditions" is meant that the nucleotide sequence specifically and/or selectively hybridizes to a target sequence (the nucleotide sequence of any of the nucleic acids described herein) in an amount that is detectably stronger than non-specific hybridization. High stringency conditions include conditions which would distinguish a polynucleotide with an exact complementary sequence, or one containing only a few scattered mismatches from a random sequence that happened to have a few small regions (*e.g.*, 3-10 bases) that matched the nucleotide sequence. Such small regions of complementarity are more easily melted than a full-length complement of 14-17 or more bases, and high stringency hybridization makes them easily distinguishable. Relatively high stringency conditions would include, for example, low salt and/or high temperature conditions, such as provided by about 0.02-0.1 M NaCl or the equivalent, at temperatures of about 50-70 °C. Such high stringency conditions tolerate little, if any, mismatch between the nucleotide sequence and the template or target strand, and are particularly suitable for detecting expression of any of the inventive TCRs. It is generally appreciated that conditions may be rendered more stringent by the addition of increasing amounts of formamide.

The present invention also provides a nucleic acid comprising a nucleotide sequence that is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to any of the nucleic acids described herein.

Typically, said nucleic acid is a DNA or RNA molecule, which may be included in a suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (*e.g.*, a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (*e.g.*, transcription and translation) of the introduced sequence. Thus, a further object encompassed by the present invention relates to a vector comprising a nucleic acid encompassed by the present invention.

Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said polypeptide upon administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40 (Mizukami T. *et al.* 1987), LTR promoter and enhancer of Moloney mouse leukemia virus (Kuwana Y *et al.* 1987), promoter (Mason J O *et al.* 1985) and enhancer (Gillies S D *et al.* 1983) of immunoglobulin H chain and the like.

Any expression vector for animal cell may be used. Examples of suitable vectors include pAGE107 (Miyaji H *et al.* 1990), pAGE103 (Mizukami T *et al.* 1987), pHSG274 (Brady G *et al.* 1984), pKCR (O'Hare K *et al.* 1981), pSG1 beta d2-4-(Miyaji H *et al.* 1990) and the like. Other representative examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like. Representative examples of viral vector include adenoviral, retroviral, lentiviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv-positive cells, 293 cells, *etc.* Detailed protocols for producing such replication-defective recombinant viruses are well-known in the art and may be found, for instance, in PCT Publ. WO 95/14785, PCT. Publ. WO 96/22378, U.S. Pat. No. 5,882,877, U.S. Pat. No. 6,013,516, U.S. Pat. No. 4,861,719, U.S. Pat. No. 5,278,056, and PCT Publ. WO 94/19478.

In some embodiments, the composition comprises an expression vector comprising an open reading frame encoding a binding protein or a polypeptide described herein or a fragment thereof. In some embodiments, the nucleic acid includes regulatory elements necessary for expression of the open reading frame. Such elements may include, for example, a promoter, an initiation codon, a stop codon, and a polyadenylation signal. In addition, enhancers may be included. These elements may be operably linked to a sequence that encodes the binding protein, polypeptide or fragment thereof.

In some embodiments, the vector further comprises nucleic acid sequence encoding CD8α, CD8β, a dominant negative TGFβ receptor (*e.g.*, a DN-TGFβRII), selectable protein marker, optionally wherein the selectable protein marker is dihydrofolate reductase (DHFR). In certain embodiments, the nucleic acid sequence encoding CD8α, CD8β, the DN-TGFβR, and/or the selectable protein marker is operably linked to a nucleic acid encoding a tag (*e.g.*, a CD34 enrichment tag). In specific embodiments, a nucleic acid sequence described herein,

such as a nucleic acid sequence encoding a TCRα, TCRβ. CD8α, CD8β, DN-TGFβR, and/or selectable protein marker are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide, such as P2A, E2A, F2A or T2A, etc.

In some embodiments, the expression vector provided herein comprises a nucleotide sequence that is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to any of the nucleic acids set forth in Tables 1-3.

As described above, representative examples of promoters include, but are not limited to, promoters from Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV) promoter, Human Immunodeficiency Virus (HIV) such as the HIV Long Terminal Repeat (LTR) promoter, Moloney virus, Cytomegalovirus (CMV) such as the CMV immediate early promoter, Epstein Barr Virus (EBV), Rous Sarcoma Virus (RSV) as well as promoters from human genes such as human actin, human myosin, human hemoglobin, human muscle creatine, and human metalothionein. Examples of suitable polyadenylation signals include but are not limited to SV40 polyadenylation signals and LTR polyadenylation signals.

In addition to the regulatory elements required for expression, other elements may also be included in the nucleic acid molecule. Such additional elements include enhancers. Enhancers include the promoters described herein. In some embodiments, enhancers/promoters include, for example, human actin, human myosin, human hemoglobin, human muscle creatine and viral enhancers such as those from CMV, RSV and EBV.

In some embodiments, the nucleic acid may be operably incorporated in a carrier or delivery vector as described further below. Useful delivery vectors include but are not limited to biodegradable microcapsules, immuno-stimulating complexes (ISCOMs) or liposomes, and genetically engineered attenuated live carriers such as viruses or bacteria.

In some embodiments, the vector is a viral vector, such as lentiviruses, retroviruses, herpes viruses, adenoviruses, adeno-associated viruses, vaccinia viruses, baculoviruses, Fowl pox, AV-pox, modified vaccinia Ankara (MVA) and other recombinant viruses. For example, a lentivirus vector may be used to infect T cells.

In some embodiments, the recombinant expression vector is capable of delivering a polynucleotide to an appropriate host cell, for example, a T cell or an antigen-presenting cell, *i.e.,* a cell that displays a peptide/MHC complex on its cell surface (*e.g.*, a dendritic cell) and lacks CD8. In some embodiments, the host cell is a hematopoietic progenitor cell or a human immune system cell. For example, the immune system cell may be a $CD4^+$ T cell, a $CD8^+$ T

cell, a CD4/CD8 double negative T cell, a gd T cell, a natural killer cell, a dendritic cell, or any combination thereof. In some embodiments, wherein a T cell is the host, the T cell may be naive, a central memory T cell, an effector memory T cell, or any combination thereof. The recombinant expression vectors may therefore also include, for example, lymphoid tissue-specific transcriptional regulatory elements (TREs), such as a B lymphocyte, T lymphocyte, or dendritic cell specific TREs. Lymphoid tissue specific TREs are known in the art (see, *e.g.*, Thompson *et al.* (1992) *Mol. Cell. Biol.* 72:1043, Todd *et al.* (1993) *J. Exp. Med.* 777:1663, and Penix *et al.* (1993) *J. Exp. Med.* 775:1483).

In some embodiments, a recombinant expression vector comprises a nucleotide sequence encoding a TCR α chain, a TCR β chain, and/or a linker peptide. For example, in some embodiments, the recombinant expression vector comprises a nucleotide sequence encoding the full-length TCR alpha and TCR beta chains of the binding protein with a linker positioned between them, wherein the nucleotide sequence encoding the beta chain is positioned 5' of the nucleotide sequence encoding the alpha chain. In some embodiments, the nucleotide sequence encodes the full-length TCR alpha and TCR beta chains with a linker positioned between them, wherein the nucleotide sequence encoding the TCR beta chain is positioned 3 ' of the nucleotide sequence encoding the TCR alpha chain. In some embodiments, the full-length TCR alpha and/or TCR beta chains are replaced with fragments thereof.

As described further below, another aspect encompassed by the present invention relates to a cell which has been transfected, infected or transformed by a nucleic acid and/or a vector in accordance with the present invention. A host cell may include any individual cell or cell culture which may receive a vector or the incorporation of nucleic acids and/or proteins, as well as any progeny cells. The term also encompasses progeny of the host cell, whether genetically or phenotypically the same or different. Suitable host cells may depend on the vector and may include mammalian cells, animal cells, human cells, simian cells, insect cells, yeast cells, and bacterial cells. These cells may be induced to incorporate the vector or other material by use of a viral vector, transformation via calcium phosphate precipitation, DEAE-dextran, electroporation, microinjection, or other methods (see, *e.g.*, Sambrook *el al.* (1989) *Molecular Cloning: A Laboratory Manual 2d ed.* (Cold Spring Harbor Laboratory)). The term "transformation" means the introduction of a "foreign" (*i.e.*, extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a

protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA has been "transformed."

The nucleic acids encompassed by the present invention may be used to produce a recombinant polypeptide encompassed by the present invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, *e.g.*, for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell.

Common expression systems include *E. coli* host cells and plasmid vectors, insect host cells and *Baculovirus* vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, *etc.*). Specific examples include *E. coli*, *Kluyveromyces* or *Saccharomyces* yeasts, mammalian cell lines (*e.g.*, Vero cells, CHO cells, 3T3 cells, COS cells, *etc.*) as well as primary or established mammalian cell cultures (*e.g.*, produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, *etc.*). Examples also include mouse SP2/0-Ag14 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G *et al* (1980), rat YB2/3HL.P2.G11.16Ag.20 cell (ATCC CRL 1662, hereinafter referred to as "YB2/0 cell"), and the like. In some embodiments, the YB2/0 cell is used since ADCC activity of chimeric or humanized binding proteins is enhanced when expressed in this cell.

The present invention also encompasses methods of producing a recombinant host cell expressing binding proteins, peptides and fragments thereof encompassed by the present invention, said method comprising the steps consisting of (i) introducing *in vitro* or *ex vivo* a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing *in vitro* or *ex vivo* the recombinant host cell obtained and (iii), optionally, selecting the cells which express said binding proteins, peptides and fragments thereof. Such recombinant host cells may be used for the diagnostic, prognostic, and/or therapeutic method encompassed by the present invention.

In another aspect, as described above, the present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment may be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides encompassed by the present invention may be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the

polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library. In some embodiments, the cDNA library comprises at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or any range in between, inclusive, such as at least about 80%-100%, full-length sequences. The cDNA libraries may be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions may optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and may be employed to identify orthologous or paralogous sequences. Optionally, polynucleotides encompassed by the present invention will encode at least a portion of a binding protein encoded by the polynucleotides described herein. The polynucleotides encompassed by the present invention embrace nucleic acid sequences that may be employed for selective hybridization to a polynucleotide encoding a binding protein encompassed by the present invention (see, *e.g.*, Ausubel, supra and Colligan, supra).

VII. Engineered cells

In an aspect encompassed by the present invention, provided herein are host cells that express proteins described herein, such as MAGEC2 immunogenic peptides, MAGEC2 immunogenic peptide-MHC (pMHC) complexes, MAGEC2 binding proteins (e.g., TCRs, antigen-binding fragments of TCRs, CARs, or fusion proteins comprising a TCR and an effector domain), and the like described herein. In some embodiments, the host cells comprise the nucleic acids or vectors described herein.

In some embodiments, a polynucleotide encoding a binding protein is used to transform, transfect, or transduce a host cell (*e.g.*, a T cell) for use in adoptive transfer therapy. Advances in nucleic acid sequencing and particular TCR sequencing have been described (*e.g.*, Robins *et al.* (2009) *Blood* 114:4099; Robins *et al.* (2010) *Sci. Translat. Med.* 2:47ra64, Robins *et al.* (2011) *J. Imm. Meth.*, and Warren *et al.* (2011) *Genome Res.* 21:790) and may be employed in the course of practicing embodiments encompassed by the present invention. Similarly, methods for transfecting or transducing T cells with desired nucleic acids are well-known in the art (*e.g.*, U.S. Pat. Publ. No. US 2004/0087025) as have adoptive transfer procedures using T cells of desired antigen-specificity (*e.g.*, Schmitt *et al.* (2009)

Hum. Gen. 20:1240, Dossett *et al.* (2009) *Mol. Ther.* 77:742, Till *et al.* (2008) *Blood* 772:2261, Wang *et al.* (2007) *Hum. Gene Ther.* 18:112, Kuball *et al.* (2007) *Blood* 709:2331, U.S. Pat. Publ. 2011/0243972, U.S. Pat. Publ. 2011/0189141, and Leen *et al.* (2007) *Ann. Rev. Immunol.* 25:243).

Any suitable immune cell may be modified to include a heterologous polynucleotide encompassed by the present invention, including, for example, a T cell, a NK cell, or a NK-T cell. In some embodiments, the cell may be a primary cell or a cell of a cell line. In some embodiments, a modified immune cell comprises a $CD4^+$T cell, a $CD8^+$ T cell, or both. For purposes herein, the T cell may be any T cell, such as a cultured T cell, *e.g.,* a primary T cell, or a T cell from a cultured T cell line, *e.g.,* Jurkat, SupTl , *etc.*, or a T cell obtained from a mammal. If obtained from a mammal, the T cell may be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells may also be enriched for or purified. In some embodiments, the T cell is a human T cell. In some embodiments, the T cell is a T cell isolated from a human. The T cell may be any type of T cell and may be of any developmental stage, including but not limited to, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic lymphocyte progenitor cell, cytotoxic lymphocyte stem cell, $CD4^+/CD8^+$ double positive T cells, $CD4^+$ helper T cells, *e.g.,* Th1 and Th2 cells, $CD4^+$ T cells, $CD8^+$ T cells (*e.g.,* cytotoxic T cells), tumor infiltrating lymphocytes (TILs), memory T cells (*e.g.,* central memory T cells and effector memory T cells), naive T cells, and the like.

Any appropriate method may be used to transfect or transduce the cells (*e.g.*, T cells), or to administer the nucleotide sequences or compositions encompassed by methods described herein. Methods for delivering polynucleotides to host cells include, for example, use of cationic polymers, lipid-like molecules, and certain commercial products such as, for example, *in vivo*-jetPEI®. Other methods include *ex vivo* transduction, injection, electroporation, DEAE-dextran, sonication loading, liposome-mediated transfection, receptor-mediated transduction, microprojectile bombardment, transposon-mediated transfer, and the like. Still further methods of transfecting or transducing host cells employ vectors, described in further detail herein.

Modified immune cells as described herein may be functionally characterized using 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 may be found, for example, in Lefkovits (Immunology Methods Manual: Hie Comprehensive Sourcebook of Techniques, 1998), as well as Current Protocols in Immunology, Weir, (1986) Handbook of Experimental Immunology, Blackwell Scientific, Boston, MA; Mishell and Shigii (eds.) (1979) Selected Methods in Cellular Immunology, Freeman Publishing, San Francisco, CA; Green and Reed (1998) *Science* 281:1309, and references cited therein.

In some embodiments, apparent affinity for a binding protein, such as a TCR or antigen-binding portion thereof, may be measured by assessing binding to various concentrations of MHC multimers. "MHC-peptide multimer staining" refers to an assay used to detect antigen-specific T cells, which, in some embodiments, 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.*, a MAGEC2 immunogenic peptide), wherein the complex is capable of binding to a binding protein, such as a TCR or antigen-binding portion thereof, that recognizes the cognate antigen. Each of the MHC molecules may be tagged with a biotin molecule. Biotinylated MHC/peptides may be multimerized (*e.g.*, tetramerized) by the addition of streptavidin, which may be fluorescently labeled.

The multimer may be detected by flow cytometry via the fluorescent label. In some embodiments, a pMHC multimer assay is used to detect or select enhanced affinity binding protein, such as a TCR or antigen-binding portion thereof, encompassed by the present invention. In some examples, apparent K_D of a binding protein, such as a TCR or antigen-binding portion thereof, is measured using 2-fold dilutions of labeled multimers 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.

Levels of cytokines may be determined using methods described herein, such as 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 Thl immune response and a Th2 immune response may be examined, for example, by determining levels of Thl cytokines, such as IFN-g, IL-12, IL-2, and TNF-b, and Type 2 cytokines, such as IL-4, IL-5, IL-9, IL-10, and IL-13.

A host cell encompassed by the present invention may comprise a single polynucleotide that encodes a binding protein as described herein, or the binding protein may be encoded by more than one polynucleotide. In other words, components or portions of a binding protein may be encoded by two or more polynucleotides, which may be contained on a single nucleic acid molecule or may be contained on two or more nucleic acid molecules.

Moreover, as described further below and in the working examples, a host ell encompassed by the present invention may encode and/or express useful accessory proteins in addition to a binding protein as described herein, either on the same polynucleotide or a different polynucleotide as the binding protein or components thereof. For example, the host cell may encode and/or express CD8α, CD8β, a DN-TGFβR (*e.g.*, a DN-TGFβRII), and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR.

In some embodiments, a polynucleotide encoding two or more components or portions of a binding protein encompassed by the present invention comprises the two or more coding sequences operatively associated in a single open reading frame. Such an arrangement can advantageously allow coordinated expression of desired gene products, such as, for example, contemporaneous expression of alpha- and beta-chains of a TCR, such that they are produced in about a 1:1 ratio. In some embodiments, two or more substituent gene products of a binding protein encompassed by the present invention, such as a TCR (*e.g.*, alpha- and beta-chains) or CAR, are expressed as separate molecules and associate post-translationally. In further embodiments, two or more substituent gene products of a binding protein encompassed by the present invention are expressed as a single peptide with the parts separated by a cleavable or removable segment. For instance, self-cleaving peptides useful for expression of separable polypeptides encoded by a single polynucleotide or vector are known in the art and include, for example, a porcine teschovirus-1 2 A (P2A) peptide, a thoseaasigna virus 2A (T2A) peptide, an equine rhinitis A virus (ERAV) 2A (E2A) peptide, and a foot-and-mouth disease vims 2A (F2A) peptide.

In some embodiments, a binding protein encompassed by the present invention comprises one or more junction amino acids. "Junction amino acids" or "junction amino acid

residues" refer to one or more (*e.g.*, 2 to about 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 can result from the design of a construct that encodes 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), or from cleavage of, for example, a self-cleaving peptide adjacent one or more domains of an encoded binding protein encompassed by the present invention (*e.g.*, a P2A peptide disposed between a TCR a-chain and a TCR β-chain, the self-cleavage of which can leave one or more junction amino acids in the a-chain, the TCR β-chain, or both).

Engineered immune cells encompassed by the present invention may be administered as therapies for, *e.g.*, a disorder characterized by MAGEC2 expression (such as a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression). In some circumstances, it may be desirable to reduce or stop the activity associated with a cellular immunotherapy. Thus, in some embodiments, an engineered immune cell encompassed by the present invention comprises a heterologous polynucleotide encoding a binding protein and an accessory protein, such as a safety switch protein, which can be targeted using a cognate drug or other compound to selectively modulate the activity (*e.g.*, lessen or ablate) of such cells when desirable. Safety switch proteins used in this regard include, for example, a truncated EGF receptor polypeptide (huEGFRt) that is devoid of extracellular N-terminal ligand binding domains and intracellular receptor tyrosine kinase activity but retains the native amino acid sequence, type I transmembrane cell surface localization, and a conformationally intact binding epitope for pharmaceutical-grade anti-EGFR monoclonal antibody, cetuximab (Erbitux) tEGF receptor (tEGFr; Wang *et al.* (2011) *Blood* 118:1255-1263), a caspase polypeptide (*e.g.*, iCasp9; Straathof *et al.* (2005) *Blood* 105:4247-4254, Di Stasi *et al.* (2011) *N. Engl. J. Med.* 365:1673-1683, Zhou and Brenner (2016) *Hematol.* pii:S0301-472X:30513-30516), RQR8 (Philip *et al.* (2014) *Blood* 124:1277-1287), and a human c-myc protein tag (Kieback *et al.* (2008) *Proc. Natl. Acad. Sci. USA* 105:623-628).

Other accessory components useful for therapeutic cells comprise a tag or selection marker (*e.g.*, a CD34 enrichment tag) that allows the cells to be identified, sorted, isolated, enriched, or tracked. For example, marked immune cells having desired characteristics (*e.g.*, an antigen-specific TCR and a safety switch protein) may be sorted away from unmarked

cells in a sample and more efficiently activated and expanded for inclusion in a therapeutic product of desired purity.

As used herein, the term "selection marker" comprises a nucleic acid construct that confers an identifiable change to a cell permitting detection and positive selection of immune cells transduced with a polynucleotide comprising a selection marker. For example, RQR is a selection marker that comprises a major extracellular loop of CD20 and two minimal CD34 binding sites. In some embodiments, an RQR-encoding polynucleotide comprises a polynucleotide that encodes the 16 amino acid CD34 minimal epitope. In some embodiments, such as certain embodiments provided in the examples herein, the CD34 minimal epitope is incorporated at the amino terminal position of the CD8 stalk domain (Q8). In further embodiments, the CD34 minimal binding site sequence may be combined with a target epitope for CD20 to form a compact marker/suicide gene for T cells (RQR8) (Philip *et al.* 2014). This construct allows for the selection of immune cells expressing the construct, with for example, CD34-specific antibody bound to magnetic beads (Miltenyi) and that utilizes clinically accepted pharmaceutical antibody, rituximab, that allows for the selective deletion of a transgene expressing engineered T cell (*e.g.*, Philip *et al.* (2014) *Blood* 124:1277-1287, U.S. Pat. Publ. 2015-0093401, and U.S. Pat. Publ. 2018-0051089).

Further exemplary selection markers include several truncated type I transmembrane proteins normally not expressed on T cells: the truncated low-affinity nerve growth factor, truncated CD19, and truncated CD34 (*e.g.*, Di Stasi *et al.* (2011) *N. Engl. J. Med.* 365:1673-1683, Mavilio *et al.* (1994) *Blood* 83:1988-1997, and Fehse *et al.* (2000) *Mol. Ther.* 7:448-456). A particularly attractive feature of CD19 and CD34 is the availability of the off-the-shelf Miltenyi CliniMACs™ selection system that can target these markers for clinical-grade sorting. However, CD19 and CD34 are relatively large surface proteins that may tax the vector packaging capacity and transcriptional efficiency of an integrating vector. Surface markers containing the extracellular, non-signaling domains or various proteins (*e.g.*, CD19, CD34, LNGFR, etc.) also may be employed. Any selection marker may be employed and should be acceptable for good manufacturing practices. In some embodiments, selection markers are expressed with a polynucleotide that encodes a gene product of interest (*e.g.*, a binding protein encompassed by the present invention, such as a TCR or CAR, or antigen-binding fragment thereof). Further examples of selection markers include, for example, reporters such as GFP, EGFP, β-gal or chloramphenicol acetyltransferase (CAT). In some embodiments, a selection marker, such as, for example, CD34 is expressed by a cell and the CD34 may be used to select enrich for, or isolate (*e.g.*, by immunomagnetic selection) the

transduced cells of interest for use in the methods described herein. As used herein, a CD34 marker is distinguished from an anti-CD34 antibody, or, for example, a scFv, TCR, or other antigen recognition moiety that binds to CD34.

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

By way of background, inclusion of $CD4^+$ T cells in an immunotherapy cell product can provide antigen-induced IL-2 secretion and augment persistence and function of transferred cytotoxic $CD8^+$ T cells (*e.g.,* Kennedy *et al.* (2008) *Immunol. Rev.* 222:129 and Nakanishi *et al. Nature* (2009) 52:510). In some embodiments, a class I-restricted TCR in $CD4^+$ T cells may require the transfer of a CD8 co-receptor to enhance sensitivity of the TCR to class I HLA peptide complexes. CD4 co-receptors differ in structure to CD8 and cannot effectively substitute for CD8 co-receptors (*e.g.,* Stone & Kranz (2013) *Front. Immunol.* 4:244 and Cole *et al.* (2012) *Immunology* 737:139). Thus, another accessory protein for use in the compositions and methods encompassed by the present invention comprises a CD8 co-receptor or component thereof. Engineered immune cells comprising a heterologous polynucleotide encoding a binding protein encompassed by the present invention may, in some embodiments, further comprise a heterologous polynucleotide encoding a CD8 co-receptor protein, or a beta-chain or alpha-chain component thereof.

A host cell may be efficiently transduced to contain, and may efficiently express, a single polynucleotide that encodes the binding protein, safety switch protein, selection marker, and CD8 co-receptor protein.

In one embodiment, the host cell encompassed by the present invention further includes a nucleic acid encoding a co-stimulatory molecule, such that the modified T cell expresses the co-stimulatory molecule. In some embodiments, the co-stimulatory domain is selected from CD3, CD27, CD28, CD83, CD86, CD127, 4-1BB, 4-1BBL, PD1 and PD1L.

In any of the foregoing embodiments, a host cell that express the binding protein described herein may be a universal immune cell. A "universal immune cell" comprises an immune cell that has been modified to reduce or eliminate expression of one or more endogenous genes that encode a polypeptide product selected from PD-l, LAG-3, CTLA4, TIM3, TIGIT, or other immune checkpoint, an HLA molecule, a TCR molecule, or any combination thereof. Without wishing to be bound by theory, certain endogenously expressed immune cell proteins may downregulate the immune activity of the modified immune cells (*e.g.,* PD-l, LAG-3, CTLA4, TIGIT), or may interfere with the binding activity

of a heterologously expressed binding protein encompassed by the present invention (*e.g.*, an endogenous TCR that binds a non-MAGEC2 antigen and interferes with the modified immune cell binding to a target cell that expresses a MAGEC2 antigen such as a MAGEC2 immunogenic peptide in the context of an MHC molecule. Further, endogenous proteins (*e.g.*, immune cell proteins, such as an HLA allele) expressed on a donor immune cell may be recognized as foreign by an allogeneic host, which may result in elimination or suppression of the modified donor immune cell by the allogeneic host.

Accordingly, decreasing or eliminating expression or activity of such endogenous genes or proteins can improve the activity, tolerance, or persistence of the modified immune 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 some embodiments, cells in accordance with the present invention are syngeneic, meaning that they are genetically identical or sufficiently identical and immunologically compatible as to allow for transplantation. In some embodiments, a universal immune cell is a donor cell (*e.g.*, allogeneic) or an autologous cell. In some embodiments, a modified immune cell (*e.g.*, a universal immune cell) encompassed by the present invention comprises a chromosomal gene knockout of one or more of a gene that encodes PD-1, LAG-3, CTLA4, TIM3, TIGIT, , or other immune checkpoint, an HLA component (*e.g.*, a gene that encodes an αl 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 *el al.* (2016) *Nature Sci. Rep.* 6:21757; Torikai *et al.* (2012) *Blood* 179:5697; and Torikai *et al.* (2013) *Blood* 722:1341, which also provide representative, exemplary gene editing techniques, compositions, and adoptive cell therapies useful according to the present invention).

As used herein, the term "chromosomal gene knockout" refers to a genetic alteration or introduced inhibitory agent in a host cell that prevents (*e.g.*, reduces, delays, suppresses, or abrogates) production, by the host cell, of a functionally active endogenous polypeptide product. Alterations resulting in a chromosomal gene knockout may 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 some embodiments, a chromosomal gene knock-out or gene knock-in may be made by chromosomal editing of a host cell. Chromosomal editing may 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 some 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 may be changed to alter triplet sequence specificity (*e.g.*, Desjarlais *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:2256-2260 and Wolfe *et al.* (1999) J. *Mol. Biol.* 255:1917-1934). Multiple zinc finger motifs may 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 some 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 well-known in the art (*e.g.,* U.S. Pat. Publ. No. US 2011/0301073, which atypical RVDs are incorporated by reference herein in their entirety). TALENs may 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 some 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 may be introduced at the site of DSB via homology directed repair. The crRNA

and tracrRNA may be engineered into a single guide RNA (sgRNA or gRNA) (*e.g.,* Jinek *et al.* (2012) *Science* 337:816-821). Further, the region of the guide RNA complementary to the target site may be altered or programed to target a desired sequence (Xie *et al.* (2014) *PLOS One* 9:e100448, U.S. Pat. Publ. No. US 2014/0068797, U.S. Pat. Publ. No. US 2014/0186843, U.S. Pat. No. 8,697,359, and PCT Publ. No. WO 2015/071474). In some 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.* (2017) *Clin. Cancer Res.* 23:2255-2266, which provides representative, exemplary gRNAs, CAS9 DNAs, vectors, and gene knockout techniques.

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 may 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, RI-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 well-known (*e.g.,* U.S. Pat. Nos. 5,420,032 and 6,833,252, Belfort *et al.* (1997) *Nucl. Acids Res.* 25:3379-3388, Dujon *et al.* (1989) *Gene* 52:115-118, Perler *et al.* (1994) *Nucl. Acids Res.* 22:1125-1127, Jasin (1996) *Trends Genet.* 72:224-228, Gimble *et al.* (1996) *J. Mol. Biol.* 263:163-180, and Argast *et al.* (1998) *J. Mol. Biol.* 280:345-353).

In some embodiments, naturally-occurring meganucleases may be used to promote site-specific genome modification of a target of interest, such as an immune checkpoint, 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.* (2005) *Nat. Biotechnol.* 23:967-73, Sussman *et al.* (2004) *J. Mol. Biol.* 342:31-41, Epinat *et al.* (2003) *Nucl. Acids Res.* 37:2952-2962, Chevalier *et al.* (2002) *Mol. Cell* 70:895-905, Ashworth *et al.* (2006) *Nature* 441:656-659, Paques *et al.* (2007) *Curr. Gene Ther.* 7:49-66, and U.S. Pat. Publ. 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 may be utilized to not only knock-out one or more target genes, but to also

introduce (knock in) heterologous or exogenous polynucleotides when used in combination with an exogenous donor template encoding a polypeptide of interest.

In some 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 binds (*e.g.*, specifically and/or selectively) to a MAGEC2 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-l, TIM3, LAG3, CTLA4, TIGIT, , or other immune checkpoint, an HLA component, or a TCR component, or any combination thereof) in the host immune cell.

A chromosomal gene knockout may be confirmed directly by DNA sequencing of the host immune cell following use of the knockout procedure or agent.

Chromosomal gene knockouts may also be inferred from the absence of gene expression (*e.g.*, the absence of an mRNA or polypeptide product encoded by the gene) following the knockout.

In some embodiments, a host cell encompassed by the present invention is capable of specifically and/or selectively killing 50% or more of target cells that comprise a peptide–MHC (pMHC) complex comprising a MAGEC2 immunogenic peptide in the context of an MHC molecule.

In some embodiments, the modified immune cell is capable of producing a cytokine when contacted with target cells that comprise a peptide–MHC (pMHC) complex comprising a MAGEC2 immunogenic peptide in the context of an MHC molecule.

In some embodiments, the cytokine comprises IFN-γ or IL2. In some embodiments, the cytokine comprises TNF-α.

In some embodiments, the host cell is capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with expression of MAGEC2 at a level of less than or equal to about 1,000 transcript per million transcripts (TPM), 950 TPM, 900 TPM, 850 TPM, 800 TPM, 750 TPM, 700 TPM, 650 TPM, 600 TPM, 550 TPM, 500 TPM, 450 TPM, 400 TPM, 350 TPM, 300 TPM, 250 TPM, 200 TPM, 150 TPM, 100 TPM, 95 TPM, 90 TPM, 85 TPM, 80 TPM, 75 TPM, 70 TPM, 65 TPM, 60 TPM, 55 TPM, 50 TPM, 45 TPM, 40 TPM, 35 TPM, 34 TPM, 33 TPM, 32 TPM, 31 TPM, 30 TPM, 29 TPM, 28 TPM, 27 TPM, 26 TPM, 25 TPM, 24 TPM, 23 TPM, 22 TPM, 21 TPM, 20 TPM, 19 TPM, 18 TPM, 17 TPM, 16 TPM, 15 TPM, 14 TPM, 13 TPM, 12 TPM, 11 TPM, 10 TPM, 9

TPM, 8 TPM, 7 TPM, 6 TPM, 5 TPM, 4 TPM, 3 TPM, 2 TPM, and 1 TPM, or any range in between, inclusive, such as less than or equal to about 1,000 TPM to less than or equal to about 35 TPM). In some embodiments, the low MAGEC2 expression level is termed "heterozygous expression" meaning between about 1 TPM and about 35 TPM, or any range in between, inclusive, such as 1-32 TPM. For example, the host cell is capable of producing an at least 1.2 fold, 1.5 fold, 1.8 fold, 2.0 fold, 2.2 fold, 2.5 fold, 2.8 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 5.5 fold, 6 fold, 6.5 fold, 7 fold, 7.5 fold, 8 fold, 8.5 fold, 9 fold, 9.5 fold, 10 fold, 11 fold, 12 fold, 13 fold, 14 fold, 15 fold, 16 fold, 17 fold, 18 fold, 19 fold, 20 fold, 25 fold, 30 fold, 35 fold, 40 fold, 45 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 1000 fold, or more, or any range in between, inclusive, such as 1.2 fold to 2 fold, higher level of cytokine or a cytotoxic molecule.

In some embodiments, the host cell is capable of specifically and/or selectively killing a taget cell expressing MAGEC2 (*e.g.*, a hyperproliferative cell expressing MAGEC2). In certain embodiments, the target cell expresses a MAGEC2 immunogenic peptide in the context of an MHC molecule (*e.g.*, a matched MHC molecule). In certain embodiments, the target cell expresses: (i) a polypeptide comprising or consisting of an amino acid sequence shown in Table 1; and (ii) a matched MHC molecule.

In some embodiments, host cells do not express MAGEC2 antigen, are not recognized by a binding protein described herein, are not of serotype HLA-B*07, and/or do not express an HLA-B*07 allele, such as HLA-B*0702. For example, a patient may receive host cells from a healthy donor who is MAGEC2-negative or negative for an MHC that presents a MAGEC2 immunogenic peptide described herein (such as HLA-B*07:02-negative), or even autologous cells that have selected and/or engineered. Cells, such as stem cells like hematopoietic stem cells, isolated from that donor (or engineered autologous cells) may be used as the source of transplant material. In parallel, T cells isolated from the same donor may be genetically engineered to recognize MAGEC2, such as by expressing a MAGEC2 binding protein described herein. Donor cells, such as stem cells, may be used to engraft cell populations into the patient (*e.g.*, hematopoietic stem cells used to reconstitute an immune system) and host cells may be infused into the patient with the goal of eliciting a highly specific anti-tumor effect. The engineered donor T cells may be designed to recognize and eliminate MAGEC-2 expressing cells, asuch as all of the patient's native blood cells, including, for example, cancer cells like residual cancer cells, which are MAGEC2-positive, thereby preventing relapse and promoting complete cures. Because a pateint's new healthy blood cells are derived from the donor and are therefore either MAGEC2-negative, HLA-

B*07 serotype-negative, and/or HLA-B*07 allele-negative (*e.g.*, negative for HLA-B*07:02), engineered cells described herein may have have minimal toxic side effects. Such patient-matched host cells and treatment methods may be used according to therapeutic methods described further below.

In some embodiments, the the killing is determined by a killing assay. In some embodiment, the killing assay is carried out by co-culturing the host cell and the target cell at a ratio from 20:1 to 0.625:1, for example, from 15:1 to 1.25:1, from 10:1 to 1.5:1, from 8:1 to 3:1, from 6:1 to 5:1, 20:1 to 5:1, 10:1 to 2.5:1 etc.. In some embodiments, the target cell is pulsed with 1 µg/mL to 50 pg/mL of MAGEC2 peptide, for example, from 1 ug/mL to 10 ng/mL, 500 ng/mL to 0.5 ng/mL, from 10 ng/mL to 10 pg/mL from 250 ng/mL to 1 ng/mL, from 50 ng/mL to 5 ng/mL, from 20 ng/mL to 10 ng/mL, etc.

In some embodiments, the host cell is capable of killing a higher number of target cells when contacted with target cells with a level of MAGEC2 less than or equal to about 1,000 transcript per million transcripts (TPM), 950 TPM, 900 TPM, 850 TPM, 800 TPM, 750 TPM, 700 TPM, 650 TPM, 600 TPM, 550 TPM, 500 TPM, 450 TPM, 400 TPM, 350 TPM, 300 TPM, 250 TPM, 200 TPM, 150 TPM, 100 TPM, 95 TPM, 90 TPM, 85 TPM, 80 TPM, 75 TPM, 70 TPM, 65 TPM, 60 TPM, 55 TPM, 50 TPM, 45 TPM, 40 TPM, 35 TPM, 34 TPM, 33 TPM, 32 TPM, 31 TPM, 30 TPM, 29 TPM, 28 TPM, 27 TPM, 26 TPM, 25 TPM, 24 TPM, 23 TPM, 22 TPM, 21 TPM, 20 TPM, 19 TPM, 18 TPM, 17 TPM, 16 TPM, 15 TPM, 14 TPM, 13 TPM, 12 TPM, 11 TPM, 10 TPM, 9 TPM, 8 TPM, 7 TPM, 6 TPM, 5 TPM, 4 TPM, 3 TPM, 2 TPM, and 1 TPM, or any range in between, inclusive, such as less than or equal to about 1,000 TPM to less than or equal to about 73 TPM). In some embodiments, the low MAGEC2 expression level is termed "heterozygous expression" meaning between about 1 TPM and about 73 TPM, or any range in between, inclusive, such as 1-73 TPM. For example, the host cell may be capable of killing an at least 1.2 fold, 1.5 fold, 1.8 fold, 2.0 fold, 2.2 fold, 2.5 fold, 2.8 fold, 3 fold, 3.5 fold, 4 fold, 4.5 fold, 5 fold, 5.5 fold, 6 fold, 6.5 fold, 7 fold, 7.5 fold, 8 fold, 8.5 fold, 9 fold, 9.5 fold, 10 fold, 11 fold, 12 fold, 13 fold, 14 fold, 15 fold, 16 fold, 17 fold, 18 fold, 19 fold, 20 fold, 25 fold, 30 fold, 35 fold, 40 fold, 45 fold, 50 fold, 60 fold, 70 fold, 80 fold, 90 fold, 100 fold, 1000 fold, or more, or any range in between, inclusive, such as 1.2 fold to 2 fold, higher number of target cells.

The present invention further provides a population of cells comprising at least one host cell described herein. The population of cells may be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, *e.g.*, a host cell (*e.g.*, a T cell), which does not comprise any

of the recombinant expression vectors, or a cell other than a T cell, *e.g.*, a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cells, a muscle cell, a brain cell, *etc.* Alternatively, the population of cells may be a substantially homogeneous population, in which the population comprises mainly of host cells (*e.g.*, consisting essentially of) comprising the recombinant expression vector. The population also may be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment encompassed by the present invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

In an embodiment encompassed by the present invention, the numbers of cells in the population may be rapidly expanded. Expansion of the numbers of T cells may be accomplished by any of a number of methods as are well-known in the art (*e.g.*, U.S. Pat. Nos. 8,034,334 and 8,383,099, U.S. Pat. Publ. No. 2012/0244133, Dudley *et al.* (2003) *J. Immunother.* 26:332-242, and Riddell *et al.* (1990) *J. Immunol. Methods* 128:189-201). For example, expansion of the numbers of T cells may be carried out by culturing the T cells with OKT3 antibody, IL-2, and feeder PBMC (*e.g.*, irradiated allogeneic PBMC).

VIII. Pharmaceutical compositions

In another aspect encompassed by the present invention, pharmaceutical compositions are provided herein comprising compositions described herein (*e.g.*, binding proteins, nucleic acids, cells, and the like) and a pharmaceutically acceptable carrier, diluent, or excipient.

The term "pharmaceutically acceptable" refers to those agents, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

Agents and other compositions encompassed by the present invention may be specially formulated for administration in solid or liquid form, including those adapted for various routes of administration, such as (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) intravaginally or

intrarectally, for example, as a pessary, cream or foam; or (5) aerosol, for example, as an aqueous aerosol, liposomal preparation or solid particles containing the compound. Any appropriate form factor for an agent or composition described herein, such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas, is contemplated.

Pharmaceutical compositions encompassed by the present invention may be presented as discrete dosage forms, such as capsules, sachets, or tablets, or liquids or aerosol sprays each containing a pre-determined amount of an active ingredient as a powder or in granules, a solution, or a suspension in an aqueous or non- aqueous liquid, an oil-in-water emulsion, a water-in-oil liquid emulsion, powders for reconstitution, powders for oral consumptions, bottles (including powders or liquids in a bottle), orally dissolving films, lozenges, pastes, tubes, gums, and packs. Such dosage forms may be prepared by any of the methods of pharmacy.

Suitable excipients include water, saline, dextrose, glycerol, or the like and combinations thereof. In some embodiments, compositions comprising host cells, binding proteins, or fusion proteins as disclosed herein further comprise a suitable infusion media. Suitable infusion media may be any isotonic medium formulation, typically normal saline, Normosol™-R (Abbott) or Plasma-Lyte™ A (Baxter), 5% dextrose in water, Ringer's lactate may be utilized. An infusion medium may be supplemented with human serum albumin or other human serum components. Unit doses comprising an effective amount of a host cell, or composition are also contemplated.

Also provided herein are unit doses that comprise an effective amount of a host cell or of a composition comprising the host cell. As described herein, host cells include immune cells, T cells ($CD4^+$ T cells and/or CD8+ T cells), cytotoxic lymphocytes (*e.g.*, cytotoxic T cells and/or natural killer (NK) cells), and the like. For example, in some embodiments, a unit dose comprises 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 cells, either alone or in combination with other cells, such as 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% other cells. In some embodiments, undesired cells are present at a reduced amount or substantially not present, such as 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 cells in the composition.

The amount of cells in a composition or unit dose is at least one cell (for example, at least one engineered $CD8^+$ T cell, engineered $CD4^+$ T cell, and/or NK cell) or is more typically greater than 10^2 cells, for example, up to 10^6, up to 10^7, up to 10^8 cells, up to 10^9 cells, or more than 10^{10} cells. In some embodiments, the cells are administered in a range from about 106 to about 10^{10} cells/m^2, such as in a range of about 10^5 to about 10^9 cells/m^2. The number of cells will depend upon the ultimate use for which the composition is intended as well the type of cells included therein. For example, cells modified to contain a binding protein specific for a particular antigen will comprise a cell population containing at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. For uses provided herein, cells are generally in a volume of a liter or less, 500 ml or less, 250 ml or less, or 100 ml or less. In embodiments, the density of the desired cells is typically greater than 10^4 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The cells may be administered as a single infusion or in multiple infusions over a range of time. A clinically relevant number of immune cells may be apportioned into multiple infusions that cumulatively equal or exceed 10^6, 10^7, 10^8, 10^9, 10^{10}, or 10^{11} cells. In some embodiments, a unit dose of the engineered immune cells may be co-administered with (*e.g.,* simultaneously or contemporaneously) hematopoietic stem cells from an allogeneic donor.

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 condition, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose and treatment regimen provide the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (such as described herein, including an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity).

An effective amount of a 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 "therapeutically effective 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 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 infusion into the patient. In some embodiments, a unit dose comprises a 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 engineered immune cells or active compound calculated to produce the desired effect in association with an appropriate pharmaceutical carrier.

In some embodiments, the pharmaceutical composition described herein and as described above for immunogenic compositions representatively exemplified for peptides, when administered to a subject, can elicit an immune response against a cell of interest that expresses MAGEC2. Such pharmaceutical compositions may be useful as vaccines for prophylactic and/or therapeutic treatment of a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression).

In some embodiments, the pharmaceutical composition further comprises a physiologically acceptable adjuvant. In some embodiments, the adjuvant employed provides for increased immunogenicity of the pharmaceutical composition. Such a further immune response stimulating compound or adjuvant may be (i) admixed to the pharmaceutical

composition in accordance with the present invention after reconstitution of the peptides and optional emulsification with an oil-based adjuvant as defined above, (ii) may be part of the reconstitution composition encompassed by the present invention defined above, (iii) may be physically linked to the peptide(s) to be reconstituted or (iv) may be administered separately to the subject, mammal or human, to be treated. The adjuvant may be one that provides for slow release of antigen (*e.g.,* the adjuvant may be a liposome), or it may be an adjuvant that is immunogenic in its own right thereby functioning synergistically with antigens. For example, the adjuvant may be a known adjuvant or other substance that promotes antigen uptake, recruits immune system cells to the site of administration, or facilitates the immune activation of responding lymphoid cells. Adjuvants include, but are not limited to, immunomodulatory molecules (*e.g.,* cytokines), oil and water emulsions, aluminum hydroxide, glucan, dextran sulfate, iron oxide, sodium alginate, bacto-adjuvant, synthetic polymers such as poly amino acids and co-polymers of amino acids, saponin, paraffin oil, and muramyl dipeptide. In some embodiments, the adjuvant is adjuvant 65, α-GalCer, aluminum phosphate, aluminum hydroxide, calcium phosphate, β-glucan peptide, CpG DNA, GM-CSF, GPI-0100, IFA, IFN-γ, IL-17, lipid A, lipopolysaccharide, Lipovant, Montanide™, N-acetyl-muramyl-L-alanyl-D-isoglutamine, pam3CSK4, quil A, trehalose dimycolate, or zymosan.

In some embodiments, the adjuvant is an immunomodulatory molecule. For example, the immunomodulatory molecule may be a recombinant protein cytokine, chemokine, or immunostimulatory agent or nucleic acid encoding cytokines, chemokines, or immunostimulatory agents designed to enhance the immunologic response.

Examples of immunomodulatory cytokines include interferons (*e.g.,* IFNα, IFNβ and IFNγ), interleukins (*e.g.,* IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-17 and IL-20), tumor necrosis factors (*e.g.,* TNFα and TNFβ), erythropoietin (EPO), FLT-3 ligand, glp10, TCA-3, MCP-1, MIF, MIP-1.alpha., MIP-1β, Rantes, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF), as well as functional fragments of any of the foregoing.

In some embodiments, an immunomodulatory chemokine that binds to a chemokine receptor, *i.e.,* a CXC, CC, C, or CX3C chemokine receptor, also may be included in the compositions provided here. Examples of chemokines include, but are not limited to, Mip1α, Mip-1β, Mip-3α (Larc), Mip-3β, Rantes, Hcc-1, Mpif-1, Mpif-2, Mcp-1, Mcp-2, Mcp-3, Mcp-4, Mcp-5, Eotaxin, Tarc, Elc, I309, IL-8, Gcp-2 Gro-α, Gro-β, Gro-γ, Nap-2, Ena-78,

Gcp-2, Ip-10, Mig, I-Tac, Sdf-1, and Bca-1 (Blc), as well as functional fragments of any of the foregoing.

In some embodiments, the composition comprises a binding protein (*e.g.*, a TCR, an antigen-binding fragment of a TCR, a CAR, or a fusion protein comprising a TCR and an effector domain), a TCRα and/or TCRβ polypeptide described herein. In some embodiments, the composition comprises a nucleic acid encoding a binding protein, a TCRα and/or TCRβ polypeptide described herein, such as a DNA molecule encoding a binding protein, a TCRα and/or TCRβ polypeptide. In some embodiments, the composition comprises an expression vector comprising an open reading frame encoding a binding protein, a TCRα and/or TCRβ polypeptide.

When taken up by a cell (*e.g.*, T cells, NK cells, *etc.*), a DNA molecule may be present in the cell as an extrachromosomal molecule and/or may integrate into the chromosome. DNA may be introduced into cells in the form of a plasmid which may remain as separate genetic material. Alternatively, linear DNAs that may integrate into the chromosome may be introduced into the cell. Optionally, when introducing DNA into a cell, reagents which promote DNA integration into chromosomes may be added.

IX. Uses and methods

The compositions described herein may be used in a variety of diagnostic, prognostic, and therapeutic applications. In any method described herein, such as a diagnostic method, prognostic method, therapeutic method, or combination thereof, all steps of the method can be performed by a single actor or, alternatively, by more than one actor. For example, diagnosis can be performed directly by the actor providing therapeutic treatment. Alternatively, a person providing a therapeutic agent can request that a diagnostic assay be performed. The diagnostician and/or the therapeutic interventionist can interpret the diagnostic assay results to determine a therapeutic strategy. Similarly, such alternative processes can apply to other assays, such as prognostic assays.

In some uses and methods encompassed by the present invention, subjects or subject samples are utilized. In some embodiments, the subject is an animal. The animal may be of either sex and may be at any stage of development. In some embodiments, the animals is a vertebrate, such as a mammal. In some embodiments, the subject is a non-human mammal. In some embodiments, the subject is a domesticated animal, such as a dog, cat, cow, pig, horse, sheep, or goat. In some embodiments, the subject is a companion animal, such as a dog or cat. In some embodiments, the subject is a livestock animal, such as a cow, pig, horse,

sheep, or goat. In some embodiments, the subject is a zoo animal. In some embodiments, the subject is a research animal, such as a rodent (*e.g.*, mouse or rat), dog, pig, or non-human primate. In some embodiments, the animal is a genetically engineered animal. In some embodiments, the animal is a transgenic animal (*e.g.*, transgenic mice and transgenic pigs). In some embodiments, the subject is a fish or reptile.

In some embodiments, the subject is a rodent, such as a mouse. In some such embodiments, the mouse is a transgenic mouse, such as a mouse expressing human MHC (*i.e.*, HLA) molecules, such as HLA-B72 (*e.g.*, Nicholson *et al.* (2012) *Adv. Hematol.* 2012:404081). In some embodiments, the subject is a transgenic mouse expressing human TCRs or is an antigen-negative mouse (*e.g.*, Li *et al.* (2010) *Nat. Med.* 16:1029-1034 and Obenaus *et al.* (2015) *Nat. Biotechnol.* 33:402-407). In some embodiments, the subject is a transgenic mouse expressing human HLA molecules and human TCRs. In some embodiments, such as where the subject is a transgenic HLA mouse, the identified TCRs are modified, *e.g.*, to be chimeric or humanized. In some embodiments, the TCR scaffold is modified, such as analogous to known binding protein humanizing methods.

In some embodiments, the subject is a human. In some embodiments, the subject is an animal model of a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, the hyperproliferative disorder, or the relapse of a hyperproliferative disorder characterized by expression of a MAGEC2 antigen). For example, the animal model may be an orthotopic xenograft animal model of a human-derived cancer.

In some embodiments, the subject is a human, such as a human with a disorder characterized by MAGEC2 expression.

The methods described herein may be used to treat a subject in need thereof. As used herein, a "subject in need thereof" includes any subject who has a disorder characterized by MAGEC2 expression, a relapse of a disorder characterized by MAGEC2 expression, and/or who is predisposed to a disorder characterized by MAGEC2 expression. As described herein, a disorder characterized by MAGEC2 expression may be a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression.

In some embodiments of the methods encompassed by the present invention, the subject has not undergone treatment for a disorder characterized by MAGEC2 expression, such as chemotherapy, radiation therapy, targeted therapy, and/or immunotherapies. In some embodiments, the subject has undergone treatment for a disorder characterized by MAGEC2 expression, such as chemotherapy, radiation therapy, targeted therapy, and/or

immunotherapies.

In some embodiments, the subject has had surgery to remove cancerous or precancerous tissue. In some embodiments, the cancerous tissue has not been removed, *e.g.*, the cancerous tissue may be located in an inoperable region of the body, such as in a tissue that is essential for life, or in a region where a surgical procedure would cause considerable risk of harm to the patient.

In some embodiments, the subject or cells thereof are resistant to a therapy of relevance, such as resistant to standard of care therapy, immune checkpoint inhibitor therapy, and the like. For example, modulating one or more biomarkers encompassed by the present invention may overcome resistance to immune checkpoint inhibitor therapy.

In some embodiments, the subjects are in need of modulation according to compositions and methods described herein, such as having been identified as having an unwanted absence, presence, or aberrant MAGEC2 expression.

a. Diagnostic methods

In an aspect encompassed by the present invention, provided herein are diagnostic methods for detecting the presence or absence of a MAGEC2 antigen, a MAGEC2 antigen-MHC complex, a cell of interest expressing MAGEC2, and/or a cell having had exposure to MAGEC2, comprising detecting the presence or absence of said MAGEC2 antigen in a sample by use of at least one binding protein, or at least one host cell described herein. In some embodiments, the method further comprising obtaining the sample (*e.g.*, from a subject). In some embodiments, the at least one binding protein or the at least one host cell, forms a complex with a MAGEC2 peptide epitope in the context of an MHC molecule, and the complex is detected in the form of fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

In an aspect encompassed by the present invention, provided herein are diagnostic methods for detecting the level of MAGEC2 or a disorder characterized by MAGEC2 expression, such as a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression, in a subject, comprising: a) contacting a sample obtained from the subject with at least one agent (*e.g.*, a MAGEC2 immunogenic peptide, MAGEC2 immunogenic peptide-MHC complex (pMHC), binding protein, host cell, or a population of host cells described herein); and b) detecting the level of reactivity, wherein a higher level of reactivity compared to a control level indicates the level

of a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression) in the subject.

In some embodiments, the level of reactivity is indicated by T cell activation or effector function, such as, but not limited to, T cell proliferation, killing, or cytokine release. The control level may be a reference number or a level of a healthy subject who has no exposure to a disorder characterized by MAGEC2 expression.

A biological sample may be obtained from a subject for determining the presence and level of an immune response to the peptide antigen (*e.g.*, a MAGEC2 antigen) 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.*, blood, isolated PBMCs, isolated T cells, lung lavage, ascites, mucosal washings, synovial fluid, etc.), 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 pharmaceutical composition, which biological sample is useful as a control for establishing baseline data.

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.

The level of an immune response, such as a cytotoxic T lymphocyte (CTL) immune response, may be determined by any one of numerous immunological methods described herein and routinely practiced in the art. For example, the level of a CTL immune response may be determined prior to and following administration of any one of the herein described 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 (*e.g.*, Henkart *el al.*, "Cytotoxic T-Lymphocytes" in Fundamental Immunology, Paul (ed.) (2003 Lippincott Williams & Wilkins, Philadelphia, PA), pages 1127-50, and references cited therein).

The present invention provides, in part, methods, systems, and code for accurately classifying whether a biological sample is associated with an output of interest, such as expression of a target of interest, such as MAGEC2. In some embodiments, the present invention is useful for classifying a sample (*e.g.*, from a subject) as associated with or at risk for responding to or not responding to therapy for a disorder characterized by MAGEC2 expression using a statistical algorithm and/or empirical data.

An exemplary method for detecting the amount or activity of MAGEC2, and thus useful for classifying whether a sample is likely or unlikely to respond to a therapy for a disorder characterized by MAGEC2 expression involves contacting a biological sample with an agent, such as a MAGEC2 immunogenic peptide or binding agent described herein, capable of detecting the amount or activity of MAGEC2 in the biological sample. In some embodiments, the method further comprise obtaining a biological sample, such as from a test subject. In some embodiments, at least one agent is used, wherein two, three, four, five, six, seven, eight, nine, ten, or more such agents may be used in combination (*e.g.*, in sandwich ELISAs) or in serial. In certain instances, the statistical algorithm is a single learning statistical classifier system. For example, a single learning statistical classifier system may be used to classify a sample as a based upon a prediction or probability value and the presence or level of the biomarker. The use of a single learning statistical classifier system typically classifies the sample with a sensitivity, specificity, positive predictive value, negative predictive value, and/or overall accuracy of at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%.

Other suitable statistical algorithms are well-known to those of skill in the art. For example, learning statistical classifier systems include a machine learning algorithmic technique capable of adapting to complex data sets (*e.g.*, panel of markers of interest) and making decisions based upon such data sets. In some embodiments, a single learning statistical classifier system such as a classification tree (*e.g.*, random forest) is used. In other embodiments, a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, or more learning statistical classifier systems are used, preferably in tandem. Examples of learning statistical classifier systems include, but are not limited to, those using inductive learning (*e.g.*, decision/classification trees such as random forests, classification and regression trees (C&RT), boosted trees, etc.), Probably Approximately Correct (PAC) learning, connectionist learning (*e.g.*, neural networks (NN), artificial neural networks (ANN), neuro fuzzy networks (NFN), network structures, perceptrons such as multi-layer perceptrons, multi-layer feed-forward networks,

applications of neural networks, Bayesian learning in belief networks, etc.), reinforcement learning (*e.g.*, passive learning in a known environment such as naive learning, adaptive dynamic learning, and temporal difference learning, passive learning in an unknown environment, active learning in an unknown environment, learning action-value functions, applications of reinforcement learning, etc.), and genetic algorithms and evolutionary programming. Other learning statistical classifier systems include support vector machines (*e.g.*, Kernel methods), multivariate adaptive regression splines (MARS), Levenberg-Marquardt algorithms, Gauss-Newton algorithms, mixtures of Gaussians, gradient descent algorithms, and learning vector quantization (LVQ). In certain embodiments, the method encompassed by the present invention further comprises sending the sample classification results to a clinician, *e.g.*, an oncologist.

In some embodiments, the diagnosis of a subject (*e.g.*, including HLA typing and/or loss of heterozygosity (LOH) to determine compatibility with TCR-HLA complex binding by TCRs of interest) is followed by administering to the individual a therapeutically effective amount of a defined treatment based upon the diagnosis.

In some embodiments, the methods further involve obtaining a control biological sample (*e.g.*, biological sample from a subject who does not have a disorder characterized by MAGEC2 expression, a subject who is in remission, a subject whose disorder is susceptible to therapy, a subject whose disorder is progressing, or other subjects of interest).

In some embodiments of analytical methods described herein, MAGEC2 expression (*e.g.*, in a sample from a subject) is compared to a pre-determined control (standard) sample. The sample from the subject is typically from a diseased tissue, such as cancer cells or tissues. The control sample may be from the same subject or from a different subject. The control sample is typically a normal, non-diseased sample. However, in some embodiments, such as for staging of disease or for evaluating the efficacy of treatment, the control sample may be from a diseased tissue. The control sample may be a combination of samples from several different subjects. In some embodiments, the MAGEC2 expression measurement(s) from a subject is compared to a pre-determined level. This pre-determined level is typically obtained from normal samples. As described herein, a "pre-determined" expression may be used to, by way of example only, evaluate a subject that may be selected for treatment, evaluate a response to cancer, and/or evaluate a response to a combination cancer therapy. A pre-determined biomarker amount and/or activity measurement(s) may be determined in populations of patients with or without a disorder characterized by MAGEC2 expression. The pre-determined biomarker amount and/or activity measurement(s) may be a single

number, equally applicable to every patient, or the pre-determined biomarker amount and/or activity measurement(s) may vary according to specific sub-populations of patients. Age, weight, height, and other factors of a subject may affect the pre-determined biomarker amount and/or activity measurement(s) of the individual. Furthermore, the pre-determined biomarker amount and/or activity may be determined for each subject individually. In one embodiment, the amounts determined and/or compared in a method described herein are based on absolute measurements.

In another embodiment, the amounts determined and/or compared in a method described herein are based on relative measurements, such as ratios (*e.g.*, biomarker copy numbers, level, and/or activity before a treatment vs. after a treatment, such biomarker measurements relative to a spiked or man-made control, such biomarker measurements relative to the expression of a housekeeping gene, and the like). For example, the relative analysis may be based on the ratio of pre-treatment biomarker measurement as compared to post-treatment biomarker measurement. Pre-treatment biomarker measurement may be made at any time prior to initiation of a therapy. Post-treatment biomarker measurement may be made at any time after initiation of therapy. In some embodiments, post-treatment biomarker measurements are made 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 weeks or more after initiation of therapy, and even longer toward indefinitely for continued monitoring. Treatment may comprise therapy to treat the disorder characterized by MAGEC2 expression, either alone or in combination with other agents, such as anti-cancer agents like chemotherapy or immune checkpoint inhibitors.

The pre-determined MAGEC2 expression may be any suitable standard. For example, the pre-determined MAGEC2 expression may be obtained from the same or a different subject for whom a subject selection is being assessed. In one embodiment, the pre-determined biomarker amount and/or activity measurement(s) may be obtained from a previous assessment of the same patient. In such a manner, the progress of the selection of the patient may be monitored over time. In addition, the control may be obtained from an assessment of another human or multiple humans, *e.g.*, selected groups of humans, if the subject is a human. In such a manner, the extent of the selection of the human for whom selection is being assessed may be compared to suitable other humans, *e.g.*, other humans who are in a similar situation to the human of interest, such as those suffering from similar or the same condition(s) and/or of the same ethnic group.

In some embodiments encompassed by the present invention the change of MAGEC2 expression from the pre-determined level is about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0,

1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 fold or greater, or any range in between, inclusive. Such cut-off values apply equally when the measurement is based on relative changes, such as based on the ratio of pre-treatment biomarker measurement as compared to post-treatment biomarker measurement.

In some embodiments, MAGEC2 expression may be detected and/or quantified by detecting or quantifying MAGEC2 polypeptide or antigen thereof, such as by using a composition described herein. The polypeptide may be detected and quantified by any of a number of means well-known to those of skill in the art, such as by immunodiffusion, immunoelectrophoresis, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, Western blotting, binder-ligand assays, immunohistochemical techniques, agglutination, complement assays, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like (*e.g.*, Basic and Clinical Immunology, Sites and Terr, eds., Appleton and Lange, Norwalk, Conn. pp 217-262, 1991).

b. Therapeutic methods

In an aspect encompassed by the present invention, provided herein are methods for preventing and/or treating a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression) and/or for inducing an immune response against a cell of interest, such as a hyperproliferative cell, expressing MAGEC2. In some embodiments, the method comprises administering to a subject a therapeutically effective amount of a composition described herein, such as an immunogenic composition, such as a composition comprising cells expressing at least one binding protein, and the like. The methods encompassed by the present invention also may be used to determine the responsiveness to cancer therapy of many different disorders characterized by MAGEC2 expression in subjects such as those described herein.

In some embodiments, the disorder characterized by MAGEC2 expression is a cancer. The terms "cancer" or "tumor" or "hyperproliferative" refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, invasive or metastatic potential, rapid growth, and certain characteristic morphological features. In some embodiments, such cells exhibit such characteristics in part or in full due to the expression and activity of immune checkpoint proteins, such as PD-1, PD-L1, PD-L2, and/or CTLA-4.

Cancer cells are often in the form of a tumor, but such cells may exist alone within an animal, or may be a non-tumorigenic cancer cell, such as in a hematologic cancer like leukemia. As used herein, the term "cancer" includes premalignant as well as malignant cancers. Cancers include, but are not limited to, a variety of cancers, carcinoma including that of the bladder (including accelerated and metastatic bladder cancer), breast, colon (including colorectal cancer), kidney, liver, lung (including small and non-small cell lung cancer and lung adenocarcinoma), ovary, prostate, testes, genitourinary tract, lymphatic system, rectum, larynx, pancreas (including exocrine pancreatic carcinoma), esophagus, stomach, gall bladder, cervix, thyroid, and skin (including squamous cell carcinoma); hematopoietic tumors of lymphoid lineage including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma, histiocytic lymphoma, and Burketts lymphoma; hematopoietic tumors of myeloid lineage including acute and chronic myelogenous leukemias, myelodysplastic syndrome, myeloid leukemia, and promyelocytic leukemia; tumors of the central and peripheral nervous system including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; other tumors including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, and teratocarcinoma; melanoma, unresectable stage III or IV malignant melanoma, squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, glioma, gastrointestinal cancer, renal cancer, ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer, prostate cancer, thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma multiforme, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, head and neck cancer, gastric cancer, germ cell tumor, bone cancer, bone tumors, adult malignant fibrous histiocytoma of bone; childhood, malignant fibrous histiocytoma of bone, sarcoma, pediatric sarcoma, sinonasal natural killer, neoplasms, plasma cell neoplasm; myelodysplastic syndromes; neuroblastoma; testicular germ cell tumor, intraocular melanoma, myelodysplastic syndromes; myelodysplastic/myeloproliferative diseases, synovial sarcoma, chronic myeloid leukemia, acute lymphoblastic leukemia, Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL), multiple myeloma, acute myelogenous leukemia, chronic lymphocytic leukemia, mastocytosis and any symptom associated with mastocytosis, and any metastasis thereof. In addition, disorders include urticaria pigmentosa, mastocytosises such as diffuse cutaneous mastocytosis, solitary mastocytoma in human, as well as dog mastocytoma and some rare subtypes like bullous,

erythrodermic and teleangiectatic mastocytosis, mastocytosis with an associated hematological disorder, such as a myeloproliferative or myelodysplastic syndrome, or acute leukemia, myeloproliferative disorder associated with mastocytosis, mast cell leukemia, in addition to other cancers. Other cancers are also included within the scope of disorders including, but are not limited to, the following: carcinoma, including that of the bladder, urothelial carcinoma, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid, testis, particularly testicular seminomas, and skin; including squamous cell carcinoma; gastrointestinal stromal tumors ("GIST"); hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Hodgkins lymphoma, non-Hodgkins lymphoma, hairy cell lymphoma and Burketts lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma;and other tumors, including melanoma, xenoderma pigmentosum, keratoactanthoma, seminoma, thyroid follicular cancer, teratocarcinoma, chemotherapy refractory non-seminomatous germ-cell tumors, and Kaposi's sarcoma, and any metastasis thereof. Other non-limiting examples of types of cancers applicable to the methods encompassed by the present invention include human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, bone cancer, brain tumor, lung carcinoma (including lung adenocarcinoma), small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, *e.g.*, acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic

(granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease. In some embodiments, cancers are epithelial in nature and include but are not limited to, bladder cancer, breast cancer, cervical cancer, colon cancer, gynecologic cancers, renal cancer, laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In some embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (*e.g.*, serous ovarian carcinoma), or breast carcinoma. The epithelial cancers may be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, Brenner, or undifferentiated. In some embodiments, the cancer is selected from the group consisting of (advanced) non-small cell lung cancer, melanoma, head and neck squamous cell cancer, (advanced) urothelial bladder cancer, (advanced) kidney cancer (RCC), microsatellite instability-high cancer, classical Hodgkin lymphoma, (advanced) gastric cancer, (advanced) cervical cancer, primary mediastinal B-cell lymphoma, (advanced) hepatocellular carcinoma, colorectal cancer, gastrointestinal cancer, breast invasive carcinoma, bladder urothelial carcinoma, and (advanced) merkel cell carcinoma.

In addition, the compositions described herein may also be administered in combination therapy to further modulate a desired activity. Additional agents include, without limitations, chemotherapeutic agents, hormones, antiangiogens, radiolabelled, compounds, or with surgery, cryotherapy, and/or radiotherapy. The preceding treatment methods may be administered in conjunction with other forms of conventional therapy (*e.g.*, standard-of-care treatments for cancer well-known to the skilled artisan), either consecutively with, pre- or post-conventional therapy. For example, these modulatory agents may be administered with a therapeutically effective dose of chemotherapeutic agent. In another embodiment, these modulatory agents are administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent. The Physicians' Desk Reference (PDR) discloses dosages of chemotherapeutic agents that have been used in the treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular melanoma, being treated, the extent of the disease and other factors familiar to the physician of skill in the art and may be determined by the physician.

Therapy using one or more compositions described herein, either alone or in combination with other therapies, such as cancer therapies, may be used to contact

MAGEC2-expressing cells and/or administered to a desired subject, such as a subject that is indicated as being a likely responder to therapy. In another embodiment, such therapy may be avoided once a subject is indicated as not being a likely responder to the therapy (*e.g.*, as assessed according to a diagnostic or prognostic method described herein) and an alternative treatment regimen, such as targeted and/or untargeted cancer therapies, may be recommended and/or administered.

The term "targeted therapy" refers to administration of agents that selectively interact with a chosen biomolecule to thereby treat cancer. For example, targeted therapy regarding the inhibition of immune checkpoint inhibitor is useful in combination with the methods encompassed by the present invention.

The term "immunotherapy" or "immunotherapies" generally refers to any strategy for modulating an immune response in a beneficial manner and encompasses the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response, as well as any treatment that uses certain parts of a subject's immune system to fight diseases, such as cancer. The subject's own immune system is stimulated (or suppressed), with or without administration of one or more agent for that purpose. Immunotherapies that are designed to elicit or amplify an immune response are referred to as "activation immunotherapies." Immunotherapies that are designed to reduce or suppress an immune response are referred to as "suppression immunotherapies." In some embodiments, an immunotherapy is specific for cells of interest, such as cancer cells. In some embodiments, immunotherapy may be "untargeted," which refers to administration of agents that do not selectively interact with immune system cells, yet modulates immune system function. Representative examples of untargeted therapies include, without limitation, chemotherapy, gene therapy, and radiation therapy.

Some forms of immunotherapy are targeted therapies that may comprise, for example, the use of cancer vaccines and/or sensitized antigen presenting cells. For example, an oncolytic virus is a virus that is able to infect and lyse cancer cells, while leaving normal cells unharmed, making them potentially useful in cancer therapy. Replication of oncolytic viruses both facilitates tumor cell destruction and also produces dose amplification at the tumor site. They may also act as vectors for anticancer genes, allowing them to be specifically delivered to the tumor site. The immunotherapy may involve passive immunity for short-term protection of a host, achieved by the administration of pre-formed antibody directed against a cancer antigen or disease antigen (*e.g.*, administration of a monoclonal antibody, optionally

linked to a chemotherapeutic agent or toxin, to a tumor antigen). Immunotherapy may also focus on using the cytotoxic lymphocyte-recognized epitopes of cancer cell lines. Alternatively, antisense polynucleotides, ribozymes, RNA interference molecules, triple helix polynucleotides and the like, may be used to selectively modulate biomolecules that are linked to the initiation, progression, and/or pathology of a tumor or cancer. Similarly, immunotherapy may take the form of cell-based therapies. For example, adoptive cellular immunotherapy is a type of immunotherapy using immune cells, such as T cells, that have a natural or genetically engineered reactivity to a patient's cancer are generated and then transferred back into the cancer patient. The injection of a large number of activated tumor-specific T cells may induce complete and durable regression of cancers.

Immunotherapy may involve passive immunity for short-term protection of a host, achieved by the administration of pre-formed antibody directed against a cancer antigen or disease antigen (*e.g.*, administration of a monoclonal antibody, optionally linked to a chemotherapeutic agent or toxin, to a tumor antigen). Immunotherapy may also focus on using the cytotoxic lymphocyte-recognized epitopes of cancer cell lines. Alternatively, antisense polynucleotides, ribozymes, RNA interference molecules, triple helix polynucleotides and the like, may be used to selectively modulate biomolecules that are linked to the initiation, progression, and/or pathology of a tumor or cancer.

In some embodiments, an immunotherapeutic agent is an agonist of an immune-stimulatory molecule; an antagonist of an immune-inhibitory molecule; an antagonist of a chemokine; an agonist of a cytokine that stimulates T cell activation; an agent that antagonizes or inhibits a cytokine that inhibits T cell activation; and/or an agent that binds to a membrane bound protein of the B7 family. In some embodiments, the immunotherapeutic agent is an antagonist of an immune-inhibitory molecule. In some embodiments, the immunotherapeutic agents may be agents for cytokines, chemokines and growth factors, for examples, neutralizing antibodies that neutralize the inhibitory effect of tumor associated cytokines, chemokines, growth factors and other soluble factors, including IL-10, TGF-β and VEGF.

In some embodiments, immunotherapy comprises inhibitors of one or more immune checkpoints. The term "immune checkpoint" refers to a group of molecules on the cell surface of CD4+ and/or CD8+ T cells that fine-tune immune responses by modulating anti-cancer immune responses, such as down-modulating or inhibiting an anti-tumor immune response. Immune checkpoint proteins are well-known in the art and include, without limitation, CTLA-4, PD-1, VISTA, B7-H2, B7-H3, PD-L1, B7-H4, B7-H6, ICOS, HVEM,

PD-L2, CD200R, CD160, gp49B, PIR-B, KRLG-1, KIR family receptors, TIM-1, TIM-3, TIM-4, LAG-3 (CD223), IDO, GITR, 4-IBB, OX-40, BTLA, SIRPalpha (CD47), CD48, 2B4 (CD244), B7.1, B7.2, ILT-2, ILT-4, TIGIT, HHLA2, butyrophilins, and A2aR (see, for example, WO 2012/177624). The term further encompasses biologically active protein fragments, as well as nucleic acids encoding full-length immune checkpoint proteins.

Some immune checkpoints are "immune-inhibitory immune checkpoints" encompassing molecules (*e.g.*, proteins) that inhibit, down-regulate, or suppress a function of the immune system (*e.g.*, an immune response). For example, PD-L1 (programmed death-ligand 1), also known as CD274 or B7-H1, is a protein that transmits an inhibitory signal that reduces proliferation of T cells to suppress the immune system. CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152, is a protein receptor on the surface of antigen-presenting cells that serves as an immune checkpoint ("off" switch) to downregulate immune responses. TIM-3 (T-cell immunoglobulin and mucin-domain containing-3), also known as HAVCR2, is a cell surface protein that serves as an immune checkpoint to regulate macrophage activation. VISTA (V-domain Ig suppressor of T cell activation) is a type I transmembrane protein that functions as an immune checkpoint to inhibit T cell effector function and maintain peripheral tolerance. LAG-3 (lymphocyte-activation gene 3) is an immune checkpoint receptor that negatively regulates proliferation, activation, and homeostasis of T cells. BTLA (B- and T-lymphocyte attenuator) is a protein that displays T cell inhibition via interactions with tumor necrosis family receptors (TNF-R). KIR (killer-cell immunoglobulin-like receptor) is a family of proteins expressed on NK cells, and a minority of T cells, that suppress the cytotoxic activity of NK cells. In some embodiments, immunotherapeutic agents may be agents specific to immunosuppressive enzymes such as inhibitors that may block the activities of arginase (ARG) and indoleamine 2,3-dioxygenase (IDO), an immune checkpoint protein that suppresses T cells and NK cells, which change the catabolism of the amino acids arginine and tryptophan in the immunosuppressive tumor microenvironment. The inhibitors may include, but are not limited to, *N*-hydroxy-L-Arg (NOHA) targeting to ARG-expressing M2 macrophages, nitroaspirin or sildenafil (Viagra®), which blocks ARG and nitric oxide synthase (NOS) simultaneously; and IDO inhibitors, such as 1-methyl-tryptophan. The term further encompasses biologically active protein fragment, as well as nucleic acids encoding full-length immune checkpoint proteins and biologically active protein fragments thereof. In some embodiment, the term further encompasses any fragment according to homology descriptions provided herein.

By contrast, other immune checkpoints are "immune-stimulatory" encompassing molecules (*e.g.*, proteins) that activate, stimulate, or promote a function of the immune system (*e.g.*, an immune response). In some embodiments, the immune-stimulatory molecule is CD28, CD80 (B7.1), CD86 (B7.2), 4-1BB (CD137), 4-1BBL (CD137L), CD27, CD70, CD40, CD40L, CD122, CD226, CD30, CD30L, OX40, OX40L, HVEM, BTLA, GITR and its ligand GITRL, LIGHT, LTβR, LTαβ, ICOS (CD278), ICOSL (B7-H2), and NKG2D. CD40 (cluster of differentiation 40) is a costimulatory protein found on antigen presenting cells that is required for their activation. OX40, also known as tumor necrosis factor receptor superfamily member 4 (TNFRSF4) or CD134, is involved in maintenance of an immune response after activation by preventing T-cell death and subsequently increasing cytokine production. CD137 is a member of the tumor necrosis factor receptor (TNF-R) family that co-stimulates activated T cells to enhance proliferation and T cell survival. CD122 is a subunit of the interleukin-2 receptor (IL-2) protein, which promotes differentiation of immature T cells into regulatory, effector, or memory T cells. CD27 is a member of the tumor necrosis factor receptor superfamily and serves as a co-stimulatory immune checkpoint molecule. CD28 (cluster of differentiation 28) is a protein expressed on T cells that provides co-stimulatory signals required for T cell activation and survival. GITR (glucocorticoid-induced TNFR-related protein), also known as TNFRSF18 and AITR, is a protein that plays a key role in dominant immunological self-tolerance maintained by regulatory T cells. ICOS (inducible T-cell co-stimulator), also known as CD278, is a CD28-superfamily costimulatory molecule that is expressed on activated T cells and play a role in T cell signaling and immune responses.

Immune checkpoints and their sequences are well-known in the art and representative embodiments are described further below. Immune checkpoints generally relate to pairs of inhibitory receptors and the natural binding partners (*e.g.*, ligands). For example, PD-1 polypeptides are inhibitory receptors capable of transmitting an inhibitory signal to an immune cell to thereby inhibit immune cell effector function, or are capable of promoting costimulation (*e.g.*, by competitive inhibition) of immune cells, *e.g.*, when present in soluble, monomeric form. Preferred PD-1 family members share sequence identity with PD-1 and bind to one or more B7 family members, *e.g.*, B7-1, B7-2, PD-1 ligand, and/or other polypeptides on antigen presenting cells. The term "PD-1 activity," includes the ability of a PD-1 polypeptide to modulate an inhibitory signal in an activated immune cell, *e.g.*, by engaging a natural PD-1 ligand on an antigen presenting cell. Modulation of an inhibitory signal in an immune cell results in modulation of proliferation of, and/or cytokine secretion

by, an immune cell. Thus, the term "PD-1 activity" includes the ability of a PD-1 polypeptide to bind its natural ligand(s), the ability to modulate immune cell inhibitory signals, and the ability to modulate the immune response. The term "PD-1 ligand" refers to binding partners of the PD-1 receptor and includes both PD-L1 (Freeman *et al.* (2000) *J. Exp. Med.* 192:1027-1034) and PD-L2 (Latchman *et al.* (2001) *Nat. Immunol.* 2:261). The term "PD-1 ligand activity" includes the ability of a PD-1 ligand polypeptide to bind its natural receptor(s) (*e.g.*, PD-1 or B7-1), the ability to modulate immune cell inhibitory signals, and the ability to modulate the immune response.

As used herein, the term "immune checkpoint therapy" refers to the use of agents that inhibit immune-inhibitory immune checkpoints, such as inhibiting their nucleic acids and/or proteins. Inhibition of one or more such immune checkpoints may block or otherwise neutralize inhibitory signaling to thereby upregulate an immune response in order to more efficaciously treat cancer. Exemplary agents useful for inhibiting immune checkpoints include antibodies, small molecules, peptides, peptidomimetics, natural ligands, and derivatives of natural ligands, that may either bind and/or inactivate or inhibit immune checkpoint proteins, or fragments thereof; as well as RNA interference, antisense, nucleic acid aptamers, etc. that may downregulate the expression and/or activity of immune checkpoint nucleic acids, or fragments thereof. Exemplary agents for upregulating an immune response include antibodies against one or more immune checkpoint proteins that block the interaction between the proteins and its natural receptor(s); a non-activating form of one or more immune checkpoint proteins (*e.g.*, a dominant negative polypeptide); small molecules or peptides that block the interaction between one or more immune checkpoint proteins and its natural receptor(s); fusion proteins (*e.g.*, the extracellular portion of an immune checkpoint inhibition protein fused to the Fc portion of an antibody or immunoglobulin) that bind to its natural receptor(s); nucleic acid molecules that block immune checkpoint nucleic acid transcription or translation; and the like. Such agents may directly block the interaction between the one or more immune checkpoints and its natural receptor(s) (*e.g.*, antibodies) to prevent inhibitory signaling and upregulate an immune response. Alternatively, agents may indirectly block the interaction between one or more immune checkpoint proteins and its natural receptor(s) to prevent inhibitory signaling and upregulate an immune response. For example, a soluble version of an immune checkpoint protein ligand such as a stabilized extracellular domain may binding to its receptor to indirectly reduce the effective concentration of the receptor to bind to an appropriate ligand. In one embodiment, anti-PD-1 antibodies, anti-PD-L1 antibodies, and/or anti-PD-L2

antibodies, either alone or in combination, are used to inhibit immune checkpoints. Therapeutic agents used for blocking the PD-1 pathway include antagonistic antibodies and soluble PD-L1 ligands. The antagonist agents against PD-1 and PD-L1/2 inhibitory pathway may include, but are not limited to, antagonistic antibodies to PD-1 or PD-L1/2 (*e.g.*, 17D8, 2D3, 4H1, 5C4 (also known as nivolumab or BMS-936558), 4A11, 7D3 and 5F4 disclosed in U.S. Pat. No. 8,008,449; AMP-224, pidilizumab (CT-011), pembrolizumab, and antibodies disclosed in U.S. Pat. Numbers 8,779,105; 8,552,154; 8,217,149; 8,168,757; 8,008,449; 7,488,802; 7,943,743; 7,635,757; and 6,808,710. Similarly, additional representative checkpoint inhibitors may be, but are not limited to, antibodies against inhibitory regulator CTLA-4 (anti-cytotoxic T-lymphocyte antigen 4 anti-cytotoxic T-lymphocyte antigen 4), such as ipilimumab, tremelimumab (fully humanized), anti-CD28 antibodies, anti-CTLA-4 adnectins, anti-CTLA-4 domain antibodies, single chain anti-CTLA-4 antibody fragments, heavy chain anti-CTLA-4 fragments, light chain anti-CTLA-4 fragments, and other antibodies, such as those disclosed in U.S. Pat. Numbers 8,748, 815; 8,529,902; 8,318,916; 8,017,114; 7,744,875; 7,605,238; 7,465,446; 7,109,003; 7,132,281; 6,984,720; 6,682,736; 6,207,156; and 5,977,318, as well as EP Pat. No. 1212422, U.S. Pat Publ. Numbers 2002/0039581 and 2002/086014, and Hurwitz *et al.* (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95:10067-10071.

The representative definitions of immune checkpoint activity, ligand, blockade, and the like exemplified for PD-1, PD-L1, PD-L2, and CTLA-4 apply generally to other immune checkpoints.

The term "untargeted therapy" refers to administration of agents that do not selectively interact with a chosen biomolecule yet treat cancer. Representative examples of untargeted therapies include, without limitation, chemotherapy, gene therapy, and radiation therapy.

In one embodiment, chemotherapy is used. Chemotherapy includes the administration of a chemotherapeutic agent. Such a chemotherapeutic agent may be, but is not limited to, those selected from among the following groups of compounds: platinum compounds, cytotoxic antibiotics, antimetabolities, anti-mitotic agents, alkylating agents, arsenic compounds, DNA topoisomerase inhibitors, taxanes, nucleoside analogues, plant alkaloids, and toxins; and synthetic derivatives thereof. Exemplary agents include, but are not limited to, alkylating agents: nitrogen mustards (*e.g.*, cyclophosphamide, ifosfamide, trofosfamide, chlorambucil, estramustine, and melphalan), nitrosoureas (*e.g.*, carmustine (BCNU) and lomustine (CCNU)), alkylsulphonates (*e.g.*, busulfan and treosulfan), triazenes

(*e.g.*, dacarbazine, temozolomide), cisplatin, treosulfan, and trofosfamide; plant alkaloids: vinblastine, paclitaxel, docetaxol; DNA topoisomerase inhibitors: teniposide, crisnatol, and mitomycin; anti-folates: methotrexate, mycophenolic acid, and hydroxyurea; pyrimidine analogs: 5-fluorouracil, doxifluridine, and cytosine arabinoside; purine analogs: mercaptopurine and thioguanine; DNA antimetabolites: 2'-deoxy-5-fluorouridine, aphidicolin glycinate, and pyrazoloimidazole; and antimitotic agents: halichondrin, colchicine, and rhizoxin. Similarly, additional exemplary agents including platinum-ontaining compounds (*e.g.*, cisplatin, carboplatin, oxaliplatin), vinca alkaloids (*e.g.*, vincristine, vinblastine, vindesine, and vinorelbine), taxoids (*e.g.*, paclitaxel or a paclitaxel equivalent such as nanoparticle albumin-bound paclitaxel (ABRAXANE), docosahexaenoic acid bound-paclitaxel (DHA-paclitaxel, Taxoprexin), polyglutamate bound-paclitaxel (PG-paclitaxel, paclitaxel poliglumex, CT-2103, XYOTAX), the tumor-activated prodrug (TAP) ANG1005 (Angiopep-2 bound to three molecules of paclitaxel), paclitaxel-EC-1 (paclitaxel bound to the erbB2-recognizing peptide EC-1), and glucose-conjugated paclitaxel, *e.g.*, 2'-paclitaxel methyl 2-glucopyranosyl succinate; docetaxel, taxol), epipodophyllins (*e.g.*, etoposide, etoposide phosphate, teniposide, topotecan, 9-aminocamptothecin, camptoirinotecan, irinotecan, crisnatol, mytomycin C), anti-metabolites, DHFR inhibitors (*e.g.*, methotrexate, dichloromethotrexate, trimetrexate, edatrexate), IMP dehydrogenase inhibitors (*e.g.*, mycophenolic acid, tiazofurin, ribavirin, and EICAR), ribonuclotide reductase inhibitors (*e.g.*, hydroxyurea and deferoxamine), uracil analogs (*e.g.*, 5-fluorouracil (5-FU), floxuridine, doxifluridine, ratitrexed, tegafur-uracil, capecitabine), cytosine analogs (*e.g.*, cytarabine (ara C), cytosine arabinoside, and fludarabine), purine analogs (*e.g.*, mercaptopurine and Thioguanine), Vitamin D3 analogs (*e.g.*, EB 1089, CB 1093, and KH 1060), isoprenylation inhibitors (*e.g.*, lovastatin), dopaminergic neurotoxins (*e.g.*, 1-methyl-4-phenylpyridinium ion), cell cycle inhibitors (*e.g.*, staurosporine), actinomycin (*e.g.*, actinomycin D, dactinomycin), bleomycin (*e.g.*, bleomycin A2, bleomycin B2, peplomycin), anthracycline (*e.g.*, daunorubicin, doxorubicin, pegylated liposomal doxorubicin, idarubicin, epirubicin, pirarubicin, zorubicin, mitoxantrone), MDR inhibitors (*e.g.*, verapamil), Ca^{2+} ATPase inhibitors (*e.g.*, thapsigargin), imatinib, thalidomide, lenalidomide, tyrosine kinase inhibitors (*e.g.*, axitinib (AG013736), bosutinib (SKI-606), cediranib (RECENTIN™, AZD2171), dasatinib (SPRYCEL®, BMS-354825), erlotinib (TARCEVA®), gefitinib (IRESSA®), imatinib (Gleevec®, CGP57148B, STI-571), lapatinib (TYKERB®, TYVERB®), lestaurtinib (CEP-701), neratinib (HKI-272), nilotinib (TASIGNA®), semaxanib (semaxinib, SU5416), sunitinib (SUTENT®, SU11248), toceranib (PALLADIA®), vandetanib

(ZACTIMA®, ZD6474), vatalanib (PTK787, PTK/ZK), trastuzumab (HERCEPTIN®), bevacizumab (AVASTIN®), rituximab (RITUXAN®), cetuximab (ERBITUX®), panitumumab (VECTIBIX®), ranibizumab (Lucentis®), nilotinib (TASIGNA®), sorafenib (NEXAVAR®), everolimus (AFINITOR®), alemtuzumab (CAMPATH®), gemtuzumab ozogamicin (MYLOTARG®), temsirolimus (TORISEL®), ENMD-2076, PCI-32765, AC220, dovitinib lactate (TKI258, CHIR-258), BIBW 2992 (TOVOK™), SGX523, PF-04217903, PF-02341066, PF-299804, BMS-777607, ABT-869, MP470, BIBF 1120 (VARGATEF®), AP24534, JNJ-26483327, MGCD265, DCC-2036, BMS-690154, CEP-11981, tivozanib (AV-951), OSI-930, MM-121, XL-184, XL-647, and/or XL228), proteasome inhibitors (*e.g.,* bortezomib (VELCADE®)), mTOR inhibitors (*e.g.,* rapamycin, temsirolimus (CCI-779), everolimus (RAD-001), ridaforolimus, AP23573 (Ariad), AZD8055 (AstraZeneca), BEZ235 (Novartis), BGT226 (Norvartis), XL765 (Sanofi Aventis), PF-4691502 (Pfizer), GDC0980 (Genentech), SF1126 (Semafoe) and OSI-027 (OSI)), oblimersen, gemcitabine, carminomycin, leucovorin, pemetrexed, cyclophosphamide, dacarbazine, procarbizine, prednisolone, dexamethasone, campathecin, plicamycin, asparaginase, aminopterin, methopterin, porfiromycin, melphalan, leurosidine, leurosine, chlorambucil, trabectedin, procarbazine, discodermolide, carminomycin,, aminopterin, and hexamethyl melamine. Compositions comprising one or more chemotherapeutic agents (*e.g.,* FLAG, CHOP) may also be used. FLAG comprises fludarabine, cytosine arabinoside (Ara-C) and G-CSF. CHOP comprises cyclophosphamide, vincristine, doxorubicin, and prednisone. In another embodiment, PARP (*e.g.,* PARP-1 and/or PARP-2) inhibitors are used and such inhibitors are well-known in the art (*e.g.,* Olaparib, ABT-888, BSI-201, BGP-15 (N-Gene Research Laboratories, Inc.); INO-1001 (Inotek Pharmaceuticals Inc.); PJ34 (Soriano *et al.*, 2001; Pacher *et al.*, 2002b); 3-aminobenzamide (Trevigen); 4-amino-1,8-naphthalimide; (Trevigen); 6(5H)-phenanthridinone (Trevigen); benzamide (U.S. Pat. Re. 36,397); and NU1025 (Bowman *et al.*). The mechanism of action is generally related to the ability of PARP inhibitors to bind PARP and decrease its activity. PARP catalyzes the conversion of beta-nicotinamide adenine dinucleotide (NAD+) into nicotinamide and poly-ADP-ribose (PAR). Both poly (ADP-ribose) and PARP have been linked to regulation of transcription, cell proliferation, genomic stability, and carcinogenesis (Bouchard et.al. (2003) *Exp. Hematol.* 31:446-454); Herceg (2001) *Mut. Res.* 477:97-110). Poly(ADP-ribose) polymerase 1 (PARP1) is a key molecule in the repair of DNA single-strand breaks (SSBs) (de Murcia J. *et al.* (1997) *Proc. Natl. Acad. Sci. U.S.A.* 94:7303-7307; Schreiber *et al.* (2006) *Nat. Rev. Mol. Cell Biol.* 7:517-528; Wang *et al.* (1997) *Genes Dev.* 11:2347-2358).

Knockout of SSB repair by inhibition of PARP1 function induces DNA double-strand breaks (DSBs) that may trigger synthetic lethality in cancer cells with defective homology-directed DSB repair (Bryant *et al.* (2005) *Nature* 434:913-917; Farmer *et al.* (2005) *Nature* 434:917-921). The foregoing examples of chemotherapeutic agents are illustrative and are not intended to be limiting.

In another embodiment, radiation therapy is used. The radiation used in radiation therapy may be ionizing radiation. Radiation therapy may also be gamma rays, X-rays, or proton beams. Examples of radiation therapy include, but are not limited to, external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. For a general overview of radiation therapy, see Hellman, Chapter 16: Principles of Cancer Management: Radiation Therapy, 6th edition, 2001, DeVita *et al.*, eds., J. B. Lippencott Company, Philadelphia. The radiation therapy may be administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. The radiation treatment may also be administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass. Also encompassed is the use of photodynamic therapy comprising the administration of photosensitizers, such as hematoporphyrin and its derivatives, Vertoporfin (BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A; and 2BA-2-DMHA.

In another embodiment, hormone therapy is used. Hormonal therapeutic treatments may comprise, for example, hormonal agonists, hormonal antagonists (*e.g.*, flutamide, bicalutamide, tamoxifen, raloxifene, leuprolide acetate (LUPRON), LH-RH antagonists), inhibitors of hormone biosynthesis and processing, and steroids (*e.g.*, dexamethasone, retinoids, deltoids, betamethasone, cortisol, cortisone, prednisone, dehydrotestosterone, glucocorticoids, mineralocorticoids, estrogen, testosterone, progestins), vitamin A derivatives (*e.g.*, all-trans retinoic acid (ATRA)); vitamin D3 analogs; antigestagens (*e.g.*, mifepristone, onapristone), or antiandrogens (*e.g.*, cyproterone acetate).

In another embodiment, hyperthermia, a procedure in which body tissue is exposed to high temperatures (up to 106°F.) is used. Heat may help shrink tumors by damaging cells or depriving them of substances they need to live. Hyperthermia therapy may be local, regional, and whole-body hyperthermia, using external and internal heating devices. Hyperthermia is almost always used with other forms of therapy (*e.g.*, radiation therapy, chemotherapy, and biological therapy) to try to increase their effectiveness. Local hyperthermia refers to heat

that is applied to a very small area, such as a tumor. The area may be heated externally with high-frequency waves aimed at a tumor from a device outside the body. To achieve internal heating, one of several types of sterile probes may be used, including thin, heated wires or hollow tubes filled with warm water; implanted microwave antennae; and radiofrequency electrodes. In regional hyperthermia, an organ or a limb is heated. Magnets and devices that produce high energy are placed over the region to be heated. In another approach, called perfusion, some of the patient's blood is removed, heated, and then pumped (perfused) into the region that is to be heated internally. Whole-body heating is used to treat metastatic cancer that has spread throughout the body. It may be accomplished using warm-water blankets, hot wax, inductive coils (like those in electric blankets), or thermal chambers (similar to large incubators). Hyperthermia does not cause any marked increase in radiation side effects or complications. Heat applied directly to the skin, however, may cause discomfort or even significant local pain in about half the patients treated. It may also cause blisters, which generally heal rapidly.

In still another embodiment, photodynamic therapy (also called PDT, photoradiation therapy, phototherapy, or photochemotherapy) is used for the treatment of some types of cancer. It is based on the discovery that certain chemicals known as photosensitizing agents may kill one-celled organisms when the organisms are exposed to a particular type of light. PDT destroys cancer cells through the use of a fixed-frequency laser light in combination with a photosensitizing agent. In PDT, the photosensitizing agent is injected into the bloodstream and absorbed by cells all over the body. The agent remains in cancer cells for a longer time than it does in normal cells. When the treated cancer cells are exposed to laser light, the photosensitizing agent absorbs the light and produces an active form of oxygen that destroys the treated cancer cells. Light exposure must be timed carefully so that it occurs when most of the photosensitizing agent has left healthy cells but is still present in the cancer cells. The laser light used in PDT may be directed through a fiber-optic (a very thin glass strand). The fiber-optic is placed close to the cancer to deliver the proper amount of light. The fiber-optic may be directed through a bronchoscope into the lungs for the treatment of lung cancer or through an endoscope into the esophagus for the treatment of esophageal cancer. An advantage of PDT is that it causes minimal damage to healthy tissue. However, because the laser light currently in use cannot pass through more than about 3 centimeters of tissue (a little more than one and an eighth inch), PDT is mainly used to treat tumors on or just under the skin or on the lining of internal organs. Photodynamic therapy makes the skin and eyes sensitive to light for 6 weeks or more after treatment. Patients are advised to avoid

direct sunlight and bright indoor light for at least 6 weeks. If patients must go outdoors, they need to wear protective clothing, including sunglasses. Other temporary side effects of PDT are related to the treatment of specific areas and may include coughing, trouble swallowing, abdominal pain, and painful breathing or shortness of breath. In December 1995, the U.S. Food and Drug Administration (FDA) approved a photosensitizing agent called porfimer sodium, or Photofrin®, to relieve symptoms of esophageal cancer that is causing an obstruction and for esophageal cancer that cannot be satisfactorily treated with lasers alone. In January 1998, the FDA approved porfimer sodium for the treatment of early nonsmall cell lung cancer in patients for whom the usual treatments for lung cancer are not appropriate. The National Cancer Institute and other institutions are supporting clinical trials (research studies) to evaluate the use of photodynamic therapy for several types of cancer, including cancers of the bladder, brain, larynx, and oral cavity.

In yet another embodiment, laser therapy is used to harness high-intensity light to destroy cancer cells. This technique is often used to relieve symptoms of cancer such as bleeding or obstruction, especially when the cancer cannot be cured by other treatments. It may also be used to treat cancer by shrinking or destroying tumors. The term "laser" stands for light amplification by stimulated emission of radiation. Ordinary light, such as that from a light bulb, has many wavelengths and spreads in all directions. Laser light, on the other hand, has a specific wavelength and is focused in a narrow beam. This type of high-intensity light contains a lot of energy. Lasers are very powerful and may be used to cut through steel or to shape diamonds. Lasers also may be used for very precise surgical work, such as repairing a damaged retina in the eye or cutting through tissue (in place of a scalpel). Although there are several different kinds of lasers, only three kinds have gained wide use in medicine: Carbon dioxide (CO_2) laser--This type of laser may remove thin layers from the skin's surface without penetrating the deeper layers. This technique is particularly useful in treating tumors that have not spread deep into the skin and certain precancerous conditions. As an alternative to traditional scalpel surgery, the CO_2 laser is also able to cut the skin. The laser is used in this way to remove skin cancers. Neodymium:yttrium-aluminum-garnet (Nd:YAG) laser-- Light from this laser may penetrate deeper into tissue than light from the other types of lasers, and it may cause blood to clot quickly. It may be carried through optical fibers to less accessible parts of the body. This type of laser is sometimes used to treat throat cancers. Argon laser--This laser may pass through only superficial layers of tissue and is therefore useful in dermatology and in eye surgery. It also is used with light-sensitive dyes to treat tumors in a procedure known as photodynamic therapy (PDT). Lasers

have several advantages over standard surgical tools, including: Lasers are more precise than scalpels. Tissue near an incision is protected, since there is little contact with surrounding skin or other tissue. The heat produced by lasers sterilizes the surgery site, thus reducing the risk of infection. Less operating time may be needed because the precision of the laser allows for a smaller incision. Healing time is often shortened; since laser heat seals blood vessels, there is less bleeding, swelling, or scarring. Laser surgery may be less complicated. For example, with fiber optics, laser light may be directed to parts of the body without making a large incision. More procedures may be done on an outpatient basis. Lasers may be used in two ways to treat cancer: by shrinking or destroying a tumor with heat, or by activating a chemical--known as a photosensitizing agent--that destroys cancer cells. In PDT, a photosensitizing agent is retained in cancer cells and may be stimulated by light to cause a reaction that kills cancer cells. CO_2 and Nd:YAG lasers are used to shrink or destroy tumors. They may be used with endoscopes, tubes that allow physicians to see into certain areas of the body, such as the bladder. The light from some lasers may be transmitted through a flexible endoscope fitted with fiber optics. This allows physicians to see and work in parts of the body that could not otherwise be reached except by surgery and therefore allows very precise aiming of the laser beam. Lasers also may be used with low-power microscopes, giving the doctor a clear view of the site being treated. Used with other instruments, laser systems may produce a cutting area as small as 200 microns in diameter--less than the width of a very fine thread. Lasers are used to treat many types of cancer. Laser surgery is a standard treatment for certain stages of glottis (vocal cord), cervical, skin, lung, vaginal, vulvar, and penile cancers. In addition to its use to destroy the cancer, laser surgery is also used to help relieve symptoms caused by cancer (palliative care). For example, lasers may be used to shrink or destroy a tumor that is blocking a patient's trachea (windpipe), making it easier to breathe. It is also sometimes used for palliation in colorectal and anal cancer. Laser-induced interstitial thermotherapy (LITT) is one of the most recent developments in laser therapy. LITT uses the same idea as a cancer treatment called hyperthermia; that heat may help shrink tumors by damaging cells or depriving them of substances they need to live. In this treatment, lasers are directed to interstitial areas (areas between organs) in the body. The laser light then raises the temperature of the tumor, which damages or destroys cancer cells.

In one aspect, provided herein is a method of eliciting in a subject an immune response to a cell that expresses MAGEC2. In some embodiments, the method comprises administering to the subject a pharmaceutical composition described herein, wherein the

pharmaceutical composition, when administered to the subject, elicits an immune response to the cell that expresses MAGEC2.

In some embodiments, the immune response can include a cell-mediated immune response. A cellular immune response is a response that involves T cells and may be determined *in vitro*, *ex vivo*, or *in vivo*. For example, a general cellular immune response may be determined as the T cell proliferative activity in cells (*e.g.*, peripheral blood leukocytes (PBLs)) sampled from the subject at a suitable time following the administering of a pharmaceutical composition. Following incubation of *e.g.*, PBMCs with a stimulator for an appropriate period, [^{3}H]thymidine incorporation may be determined. The subset of T cells that is proliferating may be determined using flow cytometry.

In another aspect encompassed by the present invention, the methods provided herein include administering to both human and non-human mammals as described above. Veterinary applications also are contemplated. In some embodiments, the subject may be any living organism in which an immune response may be elicited.

In some embodiments, the pharmaceutical composition may be administered at any time that is appropriate. For example, the administering may be conducted before or during treatment of a subject having a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression), and continued after the disorder characterized by MAGEC2 expression becomes clinically undetectable. The administering also may be continued in a subject showing signs of recurrence.

In some embodiments, the pharmaceutical composition may be administered in a therapeutically or a prophylactically effective amount. Administering the pharmaceutical composition to the subject may be carried out using known procedures, and at dosages and for periods of time sufficient to achieve a desired effect.

In some embodiments, the pharmaceutical composition may be administered to the subject at any suitable site. Administration may be accomplished using methods generally known in the art. Agents, including cells, may be introduced to the desired site by direct injection, or by any other means used in the art including, but are not limited to, intravascular, intracerebral, parenteral, intraperitoneal, intravenous, epidural, intraspinal, intrasternal, intra-articular, intra-synovial, intrathecal, intra-arterial, intracardiac, or intramuscular administration. For example, subjects of interest may be engrafted with the transplanted cells by various routes. Such routes include, but are not limited to, intravenous administration, subcutaneous administration, administration to a specific tissue (*e.g.*, focal transplantation),

injection into the femur bone marrow cavity, injection into the spleen, administration under the renal capsule of fetal liver, and the like. In certain embodiment, the cancer vaccine encompassed by the present invention is injected to the subject intratumorally or subcutaneously. Cells may be administered in one infusion, or through successive infusions over a defined time period sufficient to generate a desired effect. Exemplary methods for transplantation, engraftment assessment, and marker phenotyping analysis of transplanted cells are well-known in the art (see, for example, Pearson *et al.* (2008) *Curr. Protoc. Immunol.* 81:15.21.1-15.21.21; Ito *et al.* (2002) *Blood* 100:3175-3182; Traggiai *et al.* (2004) *Science* 304:104-107; Ishikawa *et al. Blood* (2005) 106:1565-1573; Shultz *et al.* (2005) *J. Immunol.* 174:6477-6489; and Holyoake *et al.* (1999) *Exp. Hematol.* 27:1418-1427).

In some embodiments, the dose may be administered in an amount and for a period of time effective in bringing about a desired response, be it eliciting the immune response or the prophylactic or therapeutic treatment of a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression) and/or symptoms associated therewith.

The pharmaceutical composition may be given subsequent to, preceding, or contemporaneously with other therapies including therapies that also elicit an immune response in the subject. For example, the subject may previously or concurrently be treated by other forms of immunomodulatory agents, such other therapies may be provided in such a way so as not to interfere with the immunogenicity of the compositions described herein.

Administering may be properly timed by the care giver (*e.g.*, physician, veterinarian), and may depend on the clinical condition of the subject, the objectives of administering, and/or other therapies also being contemplated or administered. In some embodiments, an initial dose may be administered, and the subject monitored for an immunological and/or clinical response. Suitable means of immunological monitoring include using patient's peripheral blood lymphocyte (PBL) as responders and immunogenic peptides or peptide-MHC complexes described herein as stimulators. An immunological reaction also may be determined by a delayed inflammatory response at the site of administering. One or more doses subsequent to the initial dose may be given as appropriate, typically on a monthly, semimonthly, or a weekly basis, until the desired effect is achieved. Thereafter, additional booster or maintenance doses may be given as required, particularly when the immunological or clinical benefit appears to subside.

In general, an appropriate dosage and treatment regimen provides the active molecules or cells in an amount sufficient to provide a benefit. Such a response may 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 viral 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.

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*, *ex vivo*, 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 an ordinarily skilled artisan.

As used herein, administration of a composition refers to delivering the same to a subject, regardless of the route or mode of delivery. Administration may be effected continuously or intermittently, and parenterally. Administration may be for treating a subject already confirmed as having a recognized condition, disease or disease state, or for treating a subject susceptible to or at risk of developing such a condition, disease or disease state. Co-administration with an adjunctive therapy may include simultaneous and/or sequential delivery of multiple agents in any order and on any dosing schedule (*e.g.*, engineered immune 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 some embodiments, a plurality of doses of a host cell (*e.g.*, an engineered immune cell) described herein is administered to the subject, which may be administered at intervals between administrations of about two to about four weeks.

Treatment or prevention methods encompassed by the present invention may be administered to a subject as part of a treatment course or regimen, which may comprise additional treatments prior to, or after, administration of the instantly disclosed unit doses, cells, or compositions. For example, in some embodiments, a subject receiving a unit dose of the host cell (*e.g.*, an engineered immune cell) is receiving or had previously received a hematopoietic cell transplant (HCT; including myeloablative and non-myeloablative HCT). In any of the foregoing embodiments, a hematopoietic cell used in an HCT may be a

"universal donor" cell that is modified to reduce or eliminate expression of one or more endogenous genes that encode a polypeptide product selected from an MHC, antigen, and a binding protein (*e.g.,* by a chromosomal gene knockout according to the methods described herein).

Techniques and regimens for performing cell transplantation are known in the art and may comprise transplantation of any suitable donor cell, such as a cell derived from umbilical cord blood, bone marrow, or peripheral blood, a hematopoietic stem cell, a mobilized stem cell, or a cell from amniotic fluid. Accordingly, in some embodiments, a host cell (*e.g.,* an engineered immune cell) encompassed by the present invention may be administered with or shortly after stem cell therapy.

Methods encompassed by the present invention may, in some embodiments, further include administering one or more additional agents to treat the disease or disorder (*e.g.,* a disorder characterized by MAGEC2 expression, such as a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression) in a combination therapy. For example, in some embodiments, a combination therapy comprises administering host cell or binding protein encompassed by the present invention with (concurrently, simultaneously, or sequentially) an antiviral agent. In some embodiments, a combination therapy comprises administering a host cell or binding protein encompassed by the present invention with lopinavir/ritonavir, chloroquine, ribavirin, steroid drugs, hydroxychloroquine, and/or interferon α. In some embodiments, a combination therapy comprises administering a host cell, composition, or unit dose of the host cells encompassed by the present invention with a secondary therapy, such as a surgery, an antibody, a vaccine, or any combination thereof.

In some embodiments, the subject is a human, such as a human with a disorder characterized by MAGEC2 expression (e.g., a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression). In some embodiments, the subject is a rodent, such as a mouse. In some such embodiments, the mouse is a transgenic mouse, such as a mouse expressing human MHC (*i.e.,* HLA) molecules, such as HLA-A2 (*e.g.*, Nicholson *et al.* (2012) *Adv. Hematol.* 2012:404081).

In some embodiments, the subject is a transgenic mouse expressing human TCRs or is an antigen-negative mouse (*e.g.*, Li *et al.* (2010) *Nat. Med.* 16:1029-1034 and Obenaus *et al.* (2015) *Nat. Biotechnol.* 33:402-407). In some embodiments, the subject is a transgenic mouse expressing human HLA molecules and human TCRs.

In some embodiments, such as where the subject is a transgenic HLA mouse, the identified TCRs are modified, *e.g.,* to be chimeric or humanized. In some embodiments, the TCR scaffold is modified, such as analogous to known binding protein humanizing methods.

c. Screening methods

Another aspect encompassed by the present invention encompasses screening assays.

In some embodiments, methods are provided for selecting agents that bind to a MAGEC2 immunogenic peptide or pMHC described herein. For example, a method of identifying a peptide-binding molecule, or antigen-binding fragment thereof, that binds to a peptide epitope selected from the peptide sequences listed in Table 1 comprising a) providing a cell presenting a peptide epitope selected from the peptide sequences listed in Table 1 in the context of an MHC molecule on the surface of the cell; b) determining binding of a plurality of candidate peptide-binding molecules or antigen-binding fragments thereof to the peptide epitope in the context of the MHC molecule on the cell; and c) identifying one or more peptide-binding molecules or antigen-binding fragments thereof that bind to the peptide epitope in the context of the MHC molecule, is provided.

In some embodiments, a method of identifying a peptide-binding molecule or antigen-binding fragment thereof that binds to a peptide epitope selected from the peptide sequences listed in Table 1 comprising: a) providing a peptide epitope either alone or in a stable MHC-peptide complex, comprising a peptide epitope selected from the peptide sequences listed in Table 1, either alone or in the context of an MHC molecule; b) determining binding of a plurality of candidate peptide-binding molecules or antigen-binding fragments thereof to the peptide or stable MHC-peptide complex; and c) identifying one or more peptide-binding molecules or antigen-binding fragments thereof that bind to the peptide epitope or the stable MHC-peptide complex, optionally wherein the MHC or MHC-peptide complex is as described herein, is provided.

In some embodiments, provide herein are methods of identifying a peptide-binding molecule or antigen-binding fragment thereof that binds to a peptide epitope selected from Table 1.

In some embodiments, the peptide binding molecule (*e.g.,* MHC-peptide binding molecule) is a molecule or portion thereof that possesses the ability to bind (*e.g.,* specifically and/or selectively) to a peptide epitope that is presented or displayed in the context of an MHC molecule (MHC-peptide complex), such as on the surface of a cell. Exemplary peptide binding molecules include T cell receptors or antibodies, or antigen-binding portions thereof,

including single chain immunoglobulin variable regions (*e.g.*, scTCR, scFv) thereof, that exhibit specific ability to bind to an MHC-peptide complex. In some embodiments, the peptide binding molecule is a TCR or antigen-binding fragment thereof. In some embodiments, the peptide binding molecule is an antibody, such as a TCR-like antibody or antigen-binding fragment thereof. In some embodiments, the peptide binding molecule is a TCR-like CAR that contains an antibody or antigen binding fragment thereof, such as a TCR-like antibody, such as one that has been engineered to bind to MHC-peptide complexes. In some embodiments, the peptide binding molecule may be derived from natural sources, or it may be partly or wholly synthetically or recombinantly produced.

In some embodiments, a binding molecule that binds to a peptide epitope may be identified by contacting one or more candidate peptide binding molecules, such as one or more candidate TCR molecules, antibodies, or antigen-binding fragments thereof, with an MHC-peptide complex, and assessing whether each of the one or more candidate binding molecules binds (*e.g.*, specifically and/or selectively) to the MHC-peptide complex. The methods may be performed *in vitro*, *ex vivo*, or *in vivo*. Methods are well-known in the art for screening, such as described in U.S. Pat. Publ. 2020/0102553.

In some embodiments, the methods include contacting a plurality or library of binding molecules, such as a plurality or library of TCRs or antibodies, with an MHC-restricted epitope and identifying or selecting molecules that specifically and/or selectively bind such an epitope. In some embodiments, a library or collection containing a plurality of different binding molecules, such as a plurality of different TCRs or a plurality of different antibodies, may be screened or assessed for binding to an MHC-restricted epitope. In some embodiments, such as for selecting a binding protein that specifically and/or selectively binds an MHC-restricted peptide, hybridoma methods may be employed.

In some embodiments, screening methods may be employed in which a plurality of candidate binding molecules, such as a library or collection of candidate binding molecules, are individually contacted with an peptide binding molecule, either simultaneously or sequentially. Library members that specifically and/or selectively bind to a particular MHC-peptide complex may be identified or selected. In some embodiments, the library or collection of candidate binding molecules may contain at least 2, 5, 10, 100, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or more different peptide binding molecules.

In some embodiments, the methods may be employed to identify a peptide binding molecule, such as a TCR or an antibody, that exhibits binding for more than one MHC haplotype or more than one MHC allele. In some embodiments, the peptide binding

molecule, such as a TCR or antibody, specifically and/or selectively binds or recognizes a peptide epitope presented in the context of a plurality of MHC class I haplotypes or alleles. In some embodiments, the peptide binding molecule, such as a TCR or antibody, specifically and/or selectively binds or recognizes a peptide epitope presented in the context of a plurality of MHC class II haplotypes or alleles.

A variety of assays are known for assessing binding affinity and/or determining whether a binding molecule specifically and/or selectively binds to a particular ligand (*e.g.*, MHC-peptide complex). It is within the level of a skilled artisan to determine the binding affinity of a TCR for a T cell epitope of a target polypeptide, such as by using any of a number of binding assays that are well-known in the art. For example, in some embodiments, a Biacore® machine may be used to determine the binding constant of a complex between two proteins. The dissociation constant (K_D) for the complex may be determined by monitoring changes in the refractive index with respect to time as buffer is passed over the chip. Other suitable assays for measuring the binding of one protein to another include, for example, immunoassays such as enzyme linked immunosorbent assays (ELISA) and radioimmunoas says (RIA), or determination of binding by monitoring the change in the spectroscopic or optical properties of the proteins through fluorescence, UV absorption, circular dichroism, or nuclear magnetic resonance (NMR). Other exemplary assays include, but are not limited to, Western blot, ELISA, analytical ultracentrifugation, spectroscopy and surface plasmon resonance (Biacore®) analysis (see, *e.g.*, Scatchard *et al.* (1949) *Ann. N.Y. Acad. Sci.* 51:660; Wilson (2002) *Science* 295:2103; Wolff *et al.* (1993) *Cancer Res.* 53:2560; and U.S. Pat. Nos. 5,283,173, 5,468,614, or the equivalent), flow cytometry, sequencing and other methods for detection of expressed nucleic acids. In one example, apparent affinity for a TCR is measured by assessing binding to various concentrations of tetramers, for example, by flow cytometry using labeled tetramers. In one example, 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.

In some embodiments, the methods may be used to identify binding molecules that bind only if the particular peptide is present in the complex, and not if the particular peptide is absent or if another, non-overlapping or unrelated peptide is present. In some embodiments, the binding molecule does not substantially bind the MHC in the absence of the bound peptide, and/or does not substantially bind the peptide in the absence of the MHC.

In some embodiments, the binding molecules are at least partially specific. In some embodiments, an exemplary identified binding molecule may bind to an MHC-peptide complex if the particular peptide is present, and also bind if a related peptide that has one or two substitutions relative to the particular peptide is present.

In some embodiments, an identified antibody, such as a TCR-like antibody, may be used to produce or generate a chimeric antigen receptors (CARs) containing a non-TCR antibody that specifically and/or selectively binds to a MHC-peptide complex.

In some embodiments, the methods of identifying a peptide binding molecule, such as a TCR or TCR-like antibody or TCR-like CAR, may be used to engineer cells expressing or containing a peptide binding molecule. In some embodiments, a cell or engineered cell is a T cell. In some embodiments, the T cell is a CD4+ or CD8+ T cell. In some embodiments, the peptide binding molecule recognizes a MHC class I peptide complex, an MHC class II peptide complex and/or an MHC-E peptide complex. In some embodiments, a peptide binding molecule, such as a TCR or antibody or CAR, that specifically and/or selectively recognizes a peptide in the context of an MHC class I may be used to engineer CD8+ T cells. In some embodiments, also provided is a composition of engineered CD8+ T cells expressing or containing the TCR, antibody or CAR, for recognition of a peptide presented in the context of MHC class I. In any of such embodiments, the cells may be used in methods of adoptive cell therapy.

In some embodiments, TCR libraries may be generated by amplification of the repertoire of Vα and Vβ from T cells isolated from a subject, including cells present in PBMCs, spleen or other lymphoid organ. In some cases, T cells may be amplified from tumor-infiltrating lymphocytes (TILs). In some embodiments, TCR libraries may be generated from CD4+ or CD8+ cells. In some embodiments, the TCRs may be amplified from a T cell source of a normal of healthy subject, *i.e.,* normal TCR libraries. In some embodiments, the TCRs may be amplified from a T cell source of a diseased subject, *i.e.,* diseased TCR libraries. In some embodiments, degenerate primers are used to amplify the gene repertoire of Vα and VP, such as by RT-PCR in samples, such as T cells, obtained from humans. In some embodiments, scTv libraries may be assembled from naive Vα and Vβ libraries in which the amplified products are cloned or assembled to be separated by a linker. Depending on the source of the subject and cells, the libraries may be HLA allele-specific.

Alternatively, in some embodiments, TCR libraries may be generated by mutagenesis or diversification of a parent or scaffold TCR molecule. For example, in some aspects, a

subject, *e.g.,* human or other mammal such as a rodent, may be vaccinated with a peptide, such as a peptide identified by the present methods. In some embodiments, a sample may be obtained from the subject, such as a sample containing blood lymphocytes. In some instances, binding molecules, *e.g.,* TCRs, may be amplified out of the sample, *e.g.,* T cells contained in the sample. In some embodiments, antigen-specific T cells may be selected, such as by screening to assess CTL activity against the peptide. In some aspects, TCRs, *e.g.,* present on the antigen-specific T cells, may be selected, such as by binding activity, *e.g.,* particular affinity or avidity for the antigen. In some aspects, the TCRs are subjected to directed evolution, such as by mutagenesis, *e.g.,* of the α or β chain. In some aspects, particular residues within CDRs of the TCR are altered. In some embodiments, selected TCRs may be modified by affinity maturation. In some aspects, a selected TCR may be used as a parent scaffold TCR against the antigen.

In some embodiments, the subject is a human, such as a human with a disorder characterized by MAGEC2 expression. In some embodiments, the subject is a rodent, such as a mouse. In some such embodiments, the mouse is a transgenic mouse, such as a mouse expressing human MHC (*i.e.,* HLA) molecules, such as HLA-A2 (*e.g.*, Nicholson *et al.* (2012) *Adv. Hematol.* 2012:404081).

In some embodiments, the subject is a transgenic mouse expressing human TCRs or is an antigen-negative mouse (*e.g.*, Li *et al.* (2010) *Nat Med.* 161029-1034; Obenaus *et al.* (2015) *Nat. Biotechnol.* 33:402-407). In some embodiments, the subject is a transgenic mouse expressing human HLA molecules and human TCRs.

In some embodiments, such as where the subject is a transgenic HLA mouse, the identified TCRs are modified, *e.g.,* to be chimeric or humanized. In some aspects, the TCR scaffold is modified, such as analogous to known antibody humanizing methods.

In some embodiments, such a scaffold molecule is used to generate a library of TCRs.

For example, in some embodiments, the library includes TCRs or antigen-binding portions thereof that have been modified or engineered compared to the parent or scaffold TCR molecule. In some embodiments, directed evolution methods may be used to generate TCRs with altered properties, such as with higher affinity for a specific MHC-peptide complex. In some embodiments, display approaches involve engineering, or modifying, a known, parent or reference TCR. For example, in some cases, a wild-type TCR may be used as a template for producing mutagenized TCRs in which in one or more residues of the CDRs are mutated, and mutants with an desired altered property, such as higher affinity for a

desired target antigen, are selected. In some embodiments, directed evolution is achieved by display methods including, but not limited to, yeast display (Holler *et al.* (2003) *Nat. Immunol.* 4:55-62; Holler *et al.* (2000) *Proc. Natl. Acad. Sci. U.S.A.* 97:5387-5392), phage display (Li *et al.* (2005) *Nat. Biotechnol.* 23:349-354), or T cell display (Chervin *et al.* (2008) *J. Immunol. Methods* 339:175-184).

In some embodiments, the libraries may be soluble. In some embodiments, the libraries are display libraries in which the TCR is displayed on the surface of a phage or cell, or attached to a particle or molecule, such as a cell, ribosome or nucleic acid, *e.g.,* RNA or DNA. Typically, the TCR libraries, including normal and disease TCR libraries or diversified libraries, may be generated in any form, including as a heterodimer or as a single chain form. In some embodiments, one or more members of the TCR may be a two-chain heterodimer. In some embodiments, pairing of the Vα and Vβ chains may be promoted by introduction of a disulfide bond. In some embodiments, members of the TCR library may be a TCR single chain (scTv or ScTCR), which, in some cases, may include a Vα and Vβ chain separated by a linker. Further, in some cases, upon screening and selection of a TCR from the library, the selected member may be generated in any form, such as a full-length TCR heterodimer or single-chain form or as antigen-binding fragments thereof.

Other methods of identifying molecules that bind to a peptide in the context of an MHC molecule are also described in U.S. Pat. Appl. No. 2020/0182884.

More generally, the present invention encompasses assays for screening agents, such as test proteins, that bind to, or modulate the activity of, MAGEC2 or an antigen thereof. Such agents include, without limitation, antibodies, proteins, fusion proteins, small molecules, and nucleic acids. In some embodiments, a method for identifying an agent which modulates an immune response entails determining the ability of the candidate agent to modulate MAGEC2 activity and further modulate an immune response of interest, such as modulated cytotoxic T cell activation and/or activity, sensitivity of cancer cells to immune checkpoint therapy, and the like.

In some embodiments, an assay is a cell-free or cell-based assay, comprising contacting a target, with a test agent, and determining the ability of the test agent to modulate (*e.g.,* upregulate or downregulate) the amount and/or activity of the target, such as by measuring direct or indirect parameters as described below.

In some embodiments, an assay is a cell-based assay, such as one comprising contacting (a) a cell of interest with a test agent and determining the ability of the test agent

to modulate the amount and/or activity of the target, such as binding characteristics. Determining the ability of the polypeptides to bind to, or interact with, each other may be accomplished, *e.g.*, by measuring direct binding or by measuring a parameter of immune cell activation or function.

In another embodiment, an assay is a cell-based assay, comprising contacting a cell such as a cancer cell with immune cells (*e.g.*, cytotoxic T cells) and a test agent, and determining the ability of the test agent to modulate the amount and/or activity of the target, and/or modulated immune responses, such as by measuring direct or indirect parameters as described below.

The methods described above and herein may also be adapted to test one or more agents that are already known to modulate the amount and/or activity of one or more biomarkers described herein to confirm modulation of the one or more biomarkers and/or to confirm the effects of the agents on readouts of a desired phenotype, such as modulated immune responses, sensitivity to immune checkpoint blockade, and the like.

In a direct binding assay, biomarker protein (or their respective target polypeptides or molecules) may be coupled with a radioisotope or enzymatic label such that binding may be determined by detecting the labeled protein or molecule in a complex. For example, the targets may be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, the targets may be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. Determining the interaction between target and substrate may also be accomplished using standard binding or enzymatic analysis assays. In one or more embodiments of the above described assay methods, it may be desirable to immobilize polypeptides or molecules to facilitate separation of complexed from uncomplexed forms of one or both of the proteins or molecules, as well as to accommodate automation of the assay.

Binding of a test agent to a target may be accomplished in any vessel suitable for containing the reactants. Non-limiting examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. Immobilized forms of the antibodies encompassed by the present invention may also include antibodies bound to a solid phase like a porous, microporous (with an average pore diameter less than about one micron) or macroporous (with an average pore diameter of more than about 10 microns) material, such as a membrane, cellulose, nitrocellulose, or glass fibers; a bead, such as that made of agarose or

polyacrylamide or latex; or a surface of a dish, plate, or well, such as one made of polystyrene.

For example, in a direct binding assay, the polypeptides may be coupled with a radioisotope or enzymatic label such that polypeptide interactions and/or activity, such as binding events, may be determined by detecting the labeled protein in a complex. For example, the polypeptides may be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, the polypeptides may be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

It is also within the scope of the present invention to determine the ability of an agent to modulate a parameter of interest without the labeling of any of the interactants. For example, a microphysiometer may be used to detect interaction between polypeptides without the labeling of polypeptides to be monitored (McConnell *et al.* (1992) *Science* 257:1906-1912). As used herein, a "microphysiometer" (*e.g.*, Cytosensor®) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate may be used as an indicator of the interaction between compound and receptor.

In some embodiments, determining the ability of a test agent (*e.g.* antibodies, fusion proteins, peptides, or small molecules) to modulate the interaction between a given set of polypeptides may be accomplished by determining the activity of one or more members of the set of polypeptides. For example, the activity of a protein and/or one or more binding partners may be determined by detecting induction of a cellular second messenger (*e.g.*, intracellular signaling), detecting catalytic/enzymatic activity of an appropriate substrate, detecting the induction of a reporter gene (comprising a target-responsive regulatory element operatively linked to a nucleic acid encoding a detectable marker, *e.g.*, chloramphenicol acetyl transferase), or detecting a cellular response regulated by the protein and/or the one or more binding partners. Determining the ability of the test agent to bind to or interact with said polypeptide may be accomplished, for example, by measuring the ability of a compound to modulate immune cell costimulation or inhibition in a proliferation assay, or by interfering with the ability of said polypeptide to bind to antibodies that recognize a portion thereof.

Agents that modulate target amount and/or activity, such as interactions with one or more binding partners, may be identified by their ability to inhibit immune cell proliferation, and/or effector function, or to induce anergy, clonal deletion, and/or exhaustion when added

to an *in vitro* assay. For example, cells may be cultured in the presence of an agent that stimulates signal transduction via an activating receptor. A number of recognized readouts of cell activation may be employed to measure, cell proliferation or effector function (*e.g.*, antibody production, cytokine production, phagocytosis) in the presence of the agent. The ability of a test agent to block this activation may be readily determined by measuring the ability of the agent to effect a decrease in proliferation or effector function being measured, using techniques known in the art.

For example, agents encompassed by the present invention may be tested for the ability to inhibit or enhance costimulation in a T cell assay, as described in Freeman *et al.* (2000) *J. Exp. Med.* 192:1027 and Latchman *et al.* (2001) *Nat. Immunol.* 2:261. CD4+ T cells may be isolated from human PBMCs and stimulated with activating anti-CD3 antibody. Proliferation of T cells may be measured by ^{3}H thymidine incorporation. An assay may be performed with or without CD28 costimulation in the assay. Similar assays may be performed with Jurkat T cells and PHA-blasts from PBMCs.

Alternatively, agents encompassed by the present invention may be tested for the ability to modulate cellular production of cytokines which are produced by or whose production is enhanced or inhibited in immune cells in response to modulation of the one or more biomarkers. Indicative cytokines released by immune cells of interest may be identified by ELISA or by the ability of an antibody which blocks the cytokine to inhibit immune cell proliferation or proliferation of other cell types that is induced by the cytokine, such as those described in the Examples section. An *in vitro* immune cell costimulation assay may also be used in a method for identifying cytokines which may be modulated by modulation of the one or more biomarkers. For example, if a particular activity induced upon costimulation, *e.g.*, immune cell proliferation, cannot be inhibited by addition of blocking antibodies to known cytokines, the activity may result from the action of an unknown cytokine. Following costimulation, this cytokine may be purified from the media by conventional methods and its activity measured by its ability to induce immune cell proliferation. To identify cytokines which may play a role the induction of tolerance, an *in vitro* T cell costimulation assay as described above may be used. In this case, T cells would be given the primary activation signal and contacted with a selected cytokine, but would not be given the costimulatory signal. After washing and resting the immune cells, the cells would be rechallenged with both a primary activation signal and a costimulatory signal. If the immune cells do not respond (*e.g.*, proliferate or produce cytokines) they have become tolerized and the cytokine has not prevented the induction of tolerance. However, if the immune cells respond,

induction of tolerance has been prevented by the cytokine. Those cytokines which are capable of preventing the induction of tolerance may be targeted for blockage *in vivo* in conjunction with reagents which block B lymphocyte antigens as a more efficient means to induce tolerance in transplant recipients or subjects with autoimmune diseases.

In some embodiments, an assay encompassed by the present invention is a cell-free assay for screening for agents that modulate the interaction between a biomarker and/or one or more binding partners, comprising contacting a polypeptide and one or more natural binding partners, or biologically active portion thereof, with a test agent and determining the ability of the test compound to modulate the interaction between the polypeptide and one or more natural binding partners, or biologically active portion thereof. Binding of the test compound may be determined either directly or indirectly as described above. In one embodiment, the assay includes contacting the polypeptide, or biologically active portion thereof, with its binding partner to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test agent to interact with the polypeptide in the assay mixture, wherein determining the ability of the test agent to interact with the polypeptide comprises determining the ability of the test agent to preferentially bind to the polypeptide or biologically active portion thereof, as compared to the binding partner.

In some embodiments, whether for cell-based or cell-free assays, a test agent may further be assayed to determine whether it affects binding and/or activity of the interaction between the polypeptide and the one or more binding partners, with other binding partners. Other useful binding analysis methods include the use of real-time Biomolecular Interaction Analysis (BIA) (Sjolander and Urbaniczky (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). As used herein, "BIA" is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, Biacore®). Changes in the optical phenomenon of surface plasmon resonance (SPR) may be used as an indication of real-time reactions between biological polypeptides. Polypeptides of interest may be immobilized on a Biacore® chip and multiple agents (blocking antibodies, fusion proteins, peptides, or small molecules) may be tested for binding to the polypeptide of interest. An example of using the BIA technology is described by Fitz *et al.* (1997) *Oncogene* 15:613.

The cell-free assays encompassed by the present invention are amenable to use of both soluble and/or membrane-bound forms of proteins. In the case of cell-free assays in which a membrane-bound form protein is used it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the protein is maintained in solution. Examples

of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton® X-100, Triton® X-114, Thesit®, Isotridecypoly(ethylene glycol ether)$_n$, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In one or more embodiments of the above described assay methods, it may be desirable to immobilize either polypeptides to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a polypeptide, may be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein may be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase-based polypeptide fusion proteins, or glutathione-S-transferase/target fusion proteins, may be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes may be dissociated from the matrix, and the level of polypeptide binding or activity determined using standard techniques.

The present invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of the present invention to further use an agent identified as described herein in an appropriate model system. For example, an agent identified as described herein may be used in a model system to determine the efficacy, toxicity, or side effects of treatment with such an agent. Alternatively, an agent identified as described herein may be used in a model system to determine the mechanism of action of such an agent. Furthermore, the present invention pertains to uses of novel agents identified by the above-described screening assays for treatments as described herein.

d. Predictive medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic

(predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect encompassed by the present invention encompasses diagnostic assays for determining (*e.g.*, detecting) the presence, absence, amount, and/or activity level of MAGEC2 or reactivity to MAGEC2 in the context of a biological sample (*e.g.*, blood, serum, cells, or tissue) to thereby determine whether an individual afflicted with a disorder characterized by MAGEC2 expression is likely to respond to therapy, whether in an original state or as a recurrence. Such assays may be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset or after recurrence of a disorder characterized by MAGEC2 expression.

The diagnostic methods described herein may furthermore be utilized to identify subjects having or at risk of developing a disorder associated with expression or lack thereof of MAGEC2. As used herein, the term "aberrant" includes an upregulation or downregulation of MAGEC2 from normal levels. Aberrant expression or activity includes increased or decreased expression or activity, as well as expression or activity which does not follow the normal developmental pattern of expression or the subcellular pattern of expression. For example, aberrant levels is intended to include the cases in which a mutation in the biomarker gene or regulatory sequence, or amplification of the chromosomal gene, thereof causes upregulation or downregulation of the biomarker of interest. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response, such as immune cell activity.

The assays described herein, such as the preceding diagnostic assays or the following assays, may be utilized to identify a subject having or at risk of developing a disorder associated with MAGEC2 misregulation. Thus, the present invention provides a method for identifying a disorder associated with aberrant or unwanted MAGEC2 regulation in which a test sample is obtained from a subject and MAGEC2 expression is detected, wherein the presence of MAGEC2 expression is diagnostic for a subject having or at risk of developing the disorder associated with aberrant or unwanted MAGEC2 expression. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample may be a biological fluid (*e.g.*, cerebrospinal fluid or serum), cell sample, or tissue, such as a histopathological slide of the tumor microenvironment, peritumoral area, and/or intratumoral area.

Furthermore, the prognostic assays described herein may be used to determine whether a subject may be administered an agent described herein to treat such a disorder associated with aberrant or unwanted MAGEC2 expression. For example, such methods may

be used to determine whether a subject may be effectively treated with one or a combination of agents. Thus, the present invention provides methods for determining whether a subject may be effectively treated with one or more agents described herein for treating a disorder associated with aberrant or unwanted MAGEC2 expression.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving the biomarker of interest.

Furthermore, any cell type or tissue in which the biomarker of interest is expressed may be utilized in the prognostic assays described herein.

e. Monitoring of effects during clinical trials

Monitoring the influence of a disorder characterized by MAGEC2 expression therapy (*e.g.*, compounds, drugs, vaccines, cell therapies, and the like) on immune responses, such as T cell reactivity (*e.g.*, the presence of binding and/or T cell activation and/or effector function), may be applied not only in basic candidate MAGEC2 antigen binding molecule screening, but also in clinical trials. For example, the effectiveness of immunogenic peptides, pMHCs, engineered cells, binding proteins, and related compositons described herein to increase an immune response (*e.g.*, T cell immune response) against cells of interest, such as hyperproliferative cells, expressing MAGEC2, may be monitored in clinical trials of subjects afflicted with a disorder characterized by MAGEC2 expression. In such clinical trials, the presence of binding and/or T cell activation and/or effector function (*e.g.*, T cell proliferation, killing, and/or cytokine release), may be used as a "read out" or marker of the phenotype of a particular cell, tissue, or system. Similarly, the effectiveness of an adaptive T cell therapy with T cells engineered to express a binding protein (*e.g.*, a TCR, an antigen-binding fragment of a TCR, a CAR, or a fusion protein comprising a TCR and an effector domain) as described herein to increase immune response to cells of interest, such as hyperproliferative cells, that are expressing MAGEC2, may be monitored in clinical trials of subjects having a disorder characterized by MAGEC2 expression. In such clinical trials, the presence of binding and/or T cell activation and/or effector function (*e.g.*, T cell proliferation, killing, or cytokine release), may be used as a "read out" or marker of the phenotype of a particular cell, tissue, or system.

In some embodiments, the present invention provides a method for monitoring the effectiveness of treatment of a therapy (*e.g.*, compounds, drugs, vaccines, cell therapies, and

the like) including the steps of a) determining the absence, presence, or level of reactivity between a sample obtained from the subject and one or more binding proteins or related composition, in a first sample obtained from the subject prior to providing at least a portion of the therapy for the disorder characterized by MAGEC2 expression to the subject, and b) determining the absence, presence, or level of reactivity between the one or more binding proteins or related composition, and a sample obtained from the subject present in a second sample obtained from the subject following provision of the portion of the therapy, wherein the presence or a higher level of reactivity in the first sample, relative to the second sample, is an indication that the therapy is efficacious for treating the disorder characterized by MAGEC2 expression in the subject and wherein the absence or a lower level of reactivity in the first sample, relative to the second sample, is an indication that the therapy is not efficacious for treating the disorder characterized by MAGEC2 expression in the subject.

In some embodiments, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (*e.g.*, antibodies, an agonist, antagonist, peptidomimetic, polypeptide, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) including the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting MAGEC2 expression in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting MAGEC2 expression in the post-administration samples; (v) comparing the MAGEC2 expresion in the pre-administration sample with the MAGEC2 expression in the post-administration sample; and (vi) altering the administration of the agent to the subject accordingly. Biomarker polypeptide analysis, such as by immunohistochemistry (IHC), may also be used to select patients who will receive therapy, such as immunotherapy.

In addition, the prognostic methods described herein may be used to determine whether a subject may be administered a therapeutic agent to treat a disorder associated with MAGEC2 expression.

f. Clinical efficacy

Clinical efficacy may be measured by any method known in the art. For example, the response to a therapy relates to any response of the disorder associated with MAGEC2 expression, *e.g.*, a tumor, to the therapy, preferably to a change in the number of cancer cells, tumor mass, and/or tumor volume, such as after initiation of neoadjuvant or adjuvant chemotherapy. Tumor response may be assessed in a neoadjuvant or adjuvant situation

where the size of a tumor after systemic intervention may be compared to the initial size and dimensions as measured by CT, PET, mammogram, ultrasound or palpation and the cellularity of a tumor may be estimated histologically and compared to the cellularity of a tumor biopsy taken before initiation of treatment. Response may also be assessed by caliper measurement or pathological examination of the tumor after biopsy or surgical resection. Response may be recorded in a quantitative fashion such as percentage change in tumor volume or cellularity or by using a semi-quantitative scoring system such as residual cancer burden (Symmans *et al.* (2007) *J. Clin. Oncol.* 25:4414-4422) or Miller-Payne score (Ogston *et al.* (2003) *Breast* (Edinburgh, Scotland) 12:320-327) in a qualitative fashion like "pathological complete response" (pCR), "clinical complete remission" (cCR), "clinical partial remission" (cPR), "clinical stable disease" (cSD), "clinical progressive disease" (cPD) or other qualitative criteria. Assessment of tumor response may be performed early after the onset of neoadjuvant or adjuvant therapy (*e.g.*, after a few hours, days, weeks or preferably after a few months). A typical endpoint for response assessment is upon termination of neoadjuvant chemotherapy or upon surgical removal of residual tumor cells and/or the tumor bed.

In some embodiments, clinical efficacy of the therapeutic treatments described herein may be determined by measuring the clinical benefit rate (CBR). The clinical benefit rate is measured by determining the sum of the percentage of patients who are in complete remission (CR), the number of patients who are in partial remission (PR) and the number of patients having stable disease (SD) at a time point at least 6 months out from the end of therapy. The shorthand for this formula is CBR=CR+PR+SD over 6 months. In some embodiments, the CBR for a particular modulator of biomarkers listed in Table 1 therapeutic regimen is at least 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, or more.

Additional criteria for evaluating the response to cancer therapy are related to "survival," which includes all of the following: survival until mortality, also known as overall survival (wherein said mortality may be either irrespective of cause or tumor related); "recurrence-free survival" (wherein the term recurrence shall include both localized and distant recurrence); metastasis free survival; disease free survival (wherein the term disease shall include cancer and diseases associated therewith). The length of said survival may be calculated by reference to a defined start point (*e.g.*, time of diagnosis or start of treatment) and end point (*e.g.*, death, recurrence, or metastasis). In addition, criteria for efficacy of

treatment may be expanded to include response to chemotherapy, probability of survival, probability of metastasis within a given time period, and probability of tumor recurrence.

For example, in order to determine appropriate threshold values, a particular agent of interest may be administered to a population of subjects and the outcome may be correlated to biomarker measurements that were determined prior to administration of any therapy. The outcome measurement may be pathologic response to therapy given in the neoadjuvant setting. Alternatively, outcome measures, such as overall survival and disease-free survival may be monitored over a period of time for subjects following therapy for whom MAGEC2 expression values are known. In certain embodiments, the same doses of the agent are administered to each subject. The period of time for which subjects are monitored may vary. For example, subjects may be monitored for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 25, 30, 35, 40, 45, 50, 55, 60 months, or longer. MAGEC2 measurement threshold values that correlate to outcome of a therapy may be determined using well-known methods, such as those described in the Examples section.

X. Cell therapy

In another aspect encompassed by the present invention, the methods include adoptive cell therapy, whereby genetically engineered cells expressing the provided molecules targeting an MHC-restricted epitope (*e.g.*, cells expressing a binding protein (*e.g.*, a TCR or CAR) or antigen-binding fragment thereof) are administered to subjects. Such administration may promote activation of immune cells (*e.g.*, T cell activation) in an antigen-targeted manner, such that the cells of interest, such as hyperproliferative cells, that express a MAGEC2 antigen are targeted for destruction.

Thus, the provided methods and uses include methods and uses for adoptive cell therapy. In some embodiments, the methods include administration of the cells or a composition containing the cells to a subject, tissue, or cell, such as one having, at risk for, or suspected of having the disease, condition or disorder. In some embodiments, the cells, populations, and compositions are administered to a subject having the particular disease or condition to be treated (*e.g.*, via adoptive cell therapy, such as by adoptive T cell therapy). In some embodiments, the cells or compositions are administered to the subject, such as a subject having or at risk for the disease or condition. In some embodiments, the methods thereby treat, *e.g.*, ameliorate one or more symptom of the disease or condition.

Methods for administration of cells for adoptive cell therapy are known and may be used in connection with the provided methods and compositions (*e.g.*, U.S. Pat. Publ. No.

2003/0170238, U.S. Pat. No. 4,690,915, Rosenberg (2011) *Nat. Rev. Clin. Oncol.* 8:577-585, Themeli *et al.* (2013) *Nat. Biotechnol.* 31:928-933, Tsukahara *et al.* (2013) *Biochem. Biophys. Res. Commun.* 438:84-89, and Davila *et al.* (2013) *PLoS ONE* 8:e61338).

In some embodiments, cell therapy (*e.g.*, adoptive cell therapy, such as adoptive T cell therapy) may be carried out by autologous transfer, in which the cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some embodiments, the cells are derived from a subject (*e.g.*, patient) in need of a treatment and the cells, following isolation and processing are administered to the same subject.

In some embodiments, the cell therapy (*e.g.*, adoptive cell therapy, such as adoptive T cell therapy) may be carried out by allogeneic transfer, in which the cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy (*e.g.*, a first subject). In such embodiments, the cells then are administered to a different subject (*e.g.*, a second subject) of the same species. In some embodiments, the first and second subjects are genetically identical (syngeneic). In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

In some embodiments, the subject, to whom the cells, cell populations, or compositions are administered is a primate, such as a human. In some embodiments, the primate is a monkey or an ape. The subject may be male or female and may be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some embodiments, the subject is a non-primate mammal, such as a rodent. In some examples, the patient or subject is a validated animal model for disease, adoptive cell therapy, and/or for assessing toxic outcomes such as cytokine release syndrome (CRS).

The binding molecules, such as TCRs, antigen-binding fragments of TCRs (*e.g.*, scTCRs) and chimeric receptors (*e.g.*, CARs) containing the TCR, and cells expressing the same, may be administered by any suitable means, for example, by injection, *e.g.*, intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjectval injection, subconjuntival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtascleral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous

administration. Dosing and administration may depend in part on whether the administration is brief or chronic. Various dosing schedules include but are not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion.

For the prevention or treatment of disease, the appropriate dosage of the binding molecule or cell may depend on the type of disease to be treated, the type of binding molecule, the severity and course of the disease, whether the binding molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the binding molecule, and the discretion of the attending physician. The compositions and molecules and cells are in some embodiments suitably administered to the patient at one time or over a series of treatments.

In some embodiments, cells may be administered at 0.1 x 10^6, 0.2 x 10^6, 0.3 x 10^6, 0.4 x 10^6, 0.5 x 10^6, 0.6 x 10^6, 0.7 x 10^6, 0.8 x 10^6, 0.9 x 10^6, 1.0 x 10^6, 5.0 x 10^6, 1.0 x 10^7, 5.0 x 10^7, 1.0 x 10^8, 5.0 x 10^8, or more, or any range in between or any value in between, cells per kilogram of subject body weight. The number of cells transplanted may be adjusted based on the desired level of engraftment in a given amount of time. Generally, 1×10^5 to about 1×10^9 cells/kg of body weight, from about 1×10^6 to about 1×10^8 cells/kg of body weight, or about 1×10^7 cells/kg of body weight, or more cells, as necessary, may be transplanted. In some embodiment, transplantation of at least about 0.1×10^6, 0.5×10^6, 1.0×10^6, 2.0×10^6, 3.0×10^6, 4.0×10^6, or 5.0×10^6 total cells relative to an average size mouse is effective. For example, in some embodiments, cells, or individual populations of sub-types of cells, may be administered to the subject at a range of about one million to about 100 billion cells and/or that amount of cells per kilogram of body weight, such as, *e.g.*, 1 million to about 50 billion cells (*e.g.*, about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (*e.g.*, about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in some cases about 100 million cells to about 50 billion cells (*e.g.*, about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges and/or per kilogram of body

weight. Dosages may vary depending on attributes particular to the disease or disorder and/or patient and/or other treatments.

Engraftment of transplanted cells may be assessed by any of various methods, such as, but not limited to, tumor volume, cytokine levels, time of administration, flow cytometric analysis of cells of interest obtained from the subject at one or more time points following transplantation, and the like. For example, a time-based analysis of waiting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28 days or may signal the time for tumor harvesting (*e.g.*, providing a dose of TCR-T cells infused 28 days apart). Any such metrics are variables that may be adjusted according to well-known parameters in order to determine the effect of the variable on a response to anti-cancer immunotherapy. In addition, the transplanted cells may be co-transplanted with other agents, such as cytokines, extracellular matrices, cell culture supports, and the like.

Cells may also be administered before, concurrently with, or after, other anti-cancer agents.

Two or more cell types may be combined and administered, such as cell-based therapy and adoptive cell transfer of stem cells, cancer vaccines and cell-based therapy, and the like. For example, adoptive cell-based immunotherapies may be combined with the cell-based therapies encompassed by the present invention. In some embodiments, the cell-based agents may be used alone or in combination with additional cell-based agents, such as immunotherapies like adoptive T cell therapy (ACT). For example, T cells genetically engineered to recognize CD19 used to treat follicular B cell lymphoma. Immune cells for ACT may be dendritic cells, T cells such as $CD8^+$ T cells and $CD4^+$ T cells, natural killer (NK) cells, NK T cells, cytotoxic T lymphocytes (CTLs), tumor infiltrating lymphocytes (TILs), lymphokine activated killer (LAK) cells, memory T cells, regulatory T cells (Tregs), helper T cells, cytokine-induced killer (CIK) cells, and any combination thereof. Well-known adoptive cell-based immunotherapeutic modalities, including, without limitation, irradiated autologous or allogeneic tumor cells, tumor lysates or apoptotic tumor cells, antigen-presenting cell-based immunotherapy, dendritic cell-based immunotherapy, adoptive T cell transfer, adoptive CAR T cell therapy, autologous immune enhancement therapy (AIET), cancer vaccines, and/or antigen presenting cells. Such cell-based immunotherapies may be further modified to express one or more gene products to further modulate immune responses, such as expressing cytokines like GM-CSF, and/or to express tumor-associated antigen (TAA) antigens, such as Mage-1, gp-100, and the like. The ratio of an agent encompassed by the present invention, such as cancer cells, to another agent encompassed by

the present invention or other composition may be 1:1 relative to each other (*e.g.*, equal amounts of 2 agents, 3 agents, 4 agents, etc.), but may modulated in any amount desired (*e.g.*, 1:1, 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, 10:1, or greater).

In some embodiments, for example, where the subject is a human, the dose includes fewer than about $1x10^8$ total binding protein (*e.g.*, TCR- or CAR-expressing cells, T cells, or peripheral blood mononuclear cells (PBMCs), *e.g.*, in the range of about $1x10^6$ to $1x10^8$ such cells, such as $2x10^6$, $5x10^6$, $1x10^7$, $5x10^7$, or $1x10^8$ or total such cells, or the range between any two of the foregoing values.

In some embodiments, the cells or related compositions described herein, such as nucleic acids, host cells, pharmaceutical formulations, and the like, may be administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as another antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent.

In some embodiments, the cells or related composition may be co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously or sequentially in any order. In some contexts, the cells or related composition are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells or related composition are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells or related composition are administered after to the one or more additional therapeutic agents.

In some embodiments, the biological activity of the cells or related composition is measured by any of a number of known methods once the cells or related composition are administered to a subject (*e.g.*, a human). Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, *in vivo*, *e.g.*, by imaging, or *in vitro*/*ex vivo*, *e.g.*, by ELISA or flow cytometry. In some embodiments, the ability of the cells to destroy target cells (cytotoxicity) may be measured using any suitable assay or method known in the art (*e.g.*, Kochenderfer *et al.* (2009) *J. Immunother.* 32: 689-702 and Herman *et al.* (2004) *J. Immunol. Meth.* 285:25-40). In some embodiments, the biological activity of the cells also may be measured by assaying expression and/or secretion of certain cytokines, such as CD107a, IFNγ, IL-2, and TNF alpha. In some embodiments, the

biological activity is measured by assessing clinical outcome, such as reduction in viral burden or load.

In some embodiments, cells are modified in any number of ways, such that their therapeutic or prophylactic efficacy is increased. For example, the binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof) expressed by the population may be conjugated either directly or indirectly through a linker to a targeting moiety. The practice of conjugating compounds to targeting moieties is well-known in the art (*e.g.*, Wadwa *et al.* (1995) *J. Drug Targeting* 3:111 and U.S. Pat. No. 5,087,616).

Immune cells, such as cytotoxic lymphocytes, may be obtained from any suitable source such as peripheral blood, spleen, and lymph nodes. The immune cells may be used as crude preparations or as partially purified or substantially purified preparations, which may be obtained by standard techniques, including, but not limited to, methods involving immunomagnetic or flow cytometry techniques using antibodies.

In certain aspects, the MAGEC2 immunogenic peptides described herein, or a nucleic acid encoding such MAGEC2 immunogenic peptides, may be used in compositions and methods for providing MAGEC2-primed, antigen-presenting cells, and/or MAGEC2-specific lymphocytes generated with these antigen-presenting cells. In some embodiments, such antigen-presenting cells and/or lymphocytes are used in the treatment and/or prevention of a disorder associated with MAGEC2 expression.

In some aspects, provided herein are methods for making MAGEC2-primed, antigen-presenting cells by contacting antigen-presenting cells with a MAGEC2 immunogenic peptide described herein, or nucleic acids encoding the at least one MAGEC2 immunogenic peptide, alone or in combination with an adjuvant, *in vitro* under a condition sufficient for the at least one MAGEC2 immunogenic polypeptide to be presented by the antigen-presenting cells.

In some embodiments, MAGEC2 immunogenic polypeptide, or nucleic acid encoding the MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant, may be contacted with a homogenous, substantially homogenous, or heterogeneous composition comprising antigen-presenting cells. For example, the composition may include but is not limited to whole blood, fresh blood, or fractions thereof such as, but not limited to, peripheral blood mononuclear cells, buffy coat fractions of whole blood, packed red cells, irradiated blood, dendritic cells, monocytes, macrophages, neutrophils, lymphocytes, natural killer cells, and natural killer T cells. If, optionally, precursors of antigen-presenting cells are used, the precursors may be cultured under suitable culture conditions sufficient to differentiate the

precursors into antigen-presenting cells. In some embodiments, the antigen-presenting cells (or precursors thereof) are selected from monocytes, macrophages, cells of myeloid lineage, B cells, dendritic cells, or Langerhans cells.

The amount of the MAGEC2 immunogenic polypeptide, or nucleic acid encoding the MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant, to be placed in contact with antigen-presenting cells may be determined by one of ordinary skill in the art by routine experimentation. Generally, antigen-presenting cells are contacted with the MAGEC2 immunogenic polypeptide, or nucleic acid encoding the MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant, for a period of time sufficient for cells to present the processed forms of the antigens for the modulation of T cells. In one embodiment, antigen-presenting cells are incubated in the presence of the MAGEC2 immunogenic polypeptide, or nucleic acid encoding the MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant, for less than about a week, illustratively, for about 1 minute to about 48 hours, about 2 minutes to about 36 hours, about 3 minutes to about 24 hours, about 4 minutes to about 12 hours, about 6 minutes to about 8 hours, about 8 minutes to about 6 hours, about 10 minutes to about 5 hours, about 15 minutes to about 4 hours, about 20 minutes to about 3 hours, about 30 minutes to about 2 hours, and about 40 minutes to about 1 hour. The time and amount of the MAGEC2 immunogenic polypeptide, or nucleic acid encoding the MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant, necessary for the antigen presenting cells to process and present the antigens may be determined, for example using pulse-chase methods wherein contact is followed by a washout period and exposure to a read-out system *e.g.*, antigen reactive T cells.

In certain embodiments, any appropriate method for delivery of antigens to the endogenous processing pathway of the antigen-presenting cells may be used. Such methods include but are not limited to, methods involving pH-sensitive liposomes, coupling of antigens to adjuvants, apoptotic cell delivery, pulsing cells onto dendritic cells, delivering recombinant chimeric virus-like particles (VLPs) comprising antigen to the MHC class I processing pathway of a dendritic cell line.

In one embodiment, solubilized MAGEC2 immunogenic polypeptide is incubated with antigen-presenting cells. In some embodiments, the MAGEC2 immunogenic polypeptide may be coupled to a cytolysin to enhance the transfer of the antigens into the cytosol of an antigen-presenting cell for delivery to the MHC class I pathway. Exemplary cytolysins include saponin compounds such as saponin-containing Immune Stimulating Complexes (ISCOM5), pore-forming toxins (*e.g.*, an alpha-toxin), and natural cytolysins of

gram-positive bacteria such as listeriolysin O (LLO), streptolysin O (SLO), and perfringolysin O (PFO).

In some embodiments, antigen-presenting cells, such as dendritic cells and macrophage, may be isolated according to methods known in the art and transfected with polynucleotides by methods known in the art for introducing a nucleic acid encoding the MAGEC2 immunogenic polypeptide into the antigen-presenting cell. Transfection reagents and methods are known in the art and commercially available. For example, RNA encoding MAGEC2 immunogenic polypeptide may be provided in a suitable medium and combined with a lipid (*e.g.*, a cationic lipid) prior to contact with antigen-presenting cells. Non-limiting examples of such lipids include LIPOFECTIN™ and LIPOFECTAMINE™. The resulting polynucleotide-lipid complex may then be contacted with antigen-presenting cells. Alternatively, the polynucleotide may be introduced into antigen-presenting cells using techniques such as electroporation or calcium phosphate transfection. The polynucleotide-loaded antigen-presenting cells may then be used to stimulate T lymphocyte (*e.g.*, cytotoxic T lymphocyte) proliferation *in vitro*, *ex vivo*, or *in vivo*. In one embodiment, the *ex vivo* expanded T lymphocyte is administered to a subject in a method of adoptive immunotherapy.

In certain aspects, provided herein is a composition comprising antigen-presenting cells that have been contacted *in vitro* with a MAGEC2 immunogenic polypeptide, or a nucleic acid encoding a MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant under a condition sufficient for a MAGEC2 immunogenic epitope to be presented by the antigen-presenting cells.

In some aspects, provided herein is a method for preparing lymphocytes specific for a MAGEC2 protein. The method comprises contacting lymphocytes with the antigen-presenting cells described above under conditions sufficient to produce a MAGEC2 protein-specific lymphocyte capable of eliciting an immune response against a cell that is infected by the MAGEC2 virus. Thus, the antigen-presenting cells also may be used to provide lymphocytes, including T lymphocytes and B lymphocytes, for eliciting an immune response against cell that is infected by the MAGEC2 virus.

In some embodiments, a preparation of T lymphocytes is contacted with the antigen-presenting cells described above for a period of time, (*e.g.*, at least about 24 hours) to priming the T lymphocytes to a MAGEC2 immunogenic epitope presented by the antigen-presenting cells.

In some embodiments, a population of antigen-presenting cells may be co-cultured with a heterogeneous population of peripheral blood T lymphocytes together with a

MAGEC2 immunogenic polypeptide, or a nucleic acid encoding a MAGEC2 immunogenic polypeptide, alone or in combination with an adjuvant. The cells may be co-cultured for a period of time and under conditions sufficient for MAGEC2 epitopes included in the MAGEC2 polypeptides to be presented by the antigen-presenting cells and the antigen-presenting cells to prime a population of T lymphocytes to respond to cells is infected by the MAGEC2 virus. In certain embodiments, provided herein are T lymphocytes and B lymphocytes that are primed to respond to cells that is infected by the MAGEC2 virus.

T lymphocytes may be obtained from any suitable source such as peripheral blood, spleen, and lymph nodes. The T lymphocytes may be used as crude preparations or as partially purified or substantially purified preparations, which may be obtained by standard techniques including, but not limited to, methods involving immunomagnetic or flow cytometry techniques using antibodies.

In certain aspects, provided herein is a composition (*e.g.*, a pharmaceutical composition) comprising the antigen-presenting cells or the lymphocytes described above, and a pharmaceutically acceptable carrier and/or diluent. In some embodiments, the composition further comprises an adjuvant as described above.

In certain aspects and as further described above, provided herein is a method for eliciting an immune response to the cell is infected by the MAGEC2 virus, the method comprising administering to the subject the antigen-presenting cells or the lymphocytes described above in effective amounts sufficient to elicit the immune response. In some embodiments, provided herein is a method for treatment or prophylaxis of a disorder characterized by MAGEC2 expression, the method comprising administering to the subject an effective amount of the antigen-presenting cells or the lymphocytes described above. In one embodiment, the antigen-presenting cells or the lymphocytes are administered systemically, preferably by injection. Alternately, one may administer locally rather than systemically, for example, via injection directly into tissue, preferably in a depot or sustained release formulation.

In certain embodiments, the antigen-primed antigen-presenting cells described herein and the antigen-specific T lymphocytes generated with these antigen-presenting cells may be used as active compounds in immunomodulating compositions for prophylactic or therapeutic treatment of a disorder characterized by MAGEC2 expression. In some embodiments, the MAGEC2 -primed antigen-presenting cells described herein may be used for generating $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, and/or B lymphocytes for adoptive transfer to the subject. Thus, for example, MAGEC2 -specific lymphocyte may be adoptively

transferred for therapeutic purposes in subjects afflicted with a disorder characterized by MAGEC2 expression.

In certain embodiments, the antigen-presenting cells and/or lymphocytes described herein may be administered to a subject, either by themselves or in combination, for eliciting an immune response, particularly for eliciting an immune response to cells expressing MAGEC2. In some embodiments, the antigen-presenting cells and/or lymphocytes may be derived from the subject (*i.e.*, autologous cells) or from a different subject that is MHC matched or mismatched with the subject (*e.g.*, allogeneic).

Single or multiple administrations of the antigen-presenting cells and lymphocytes may be carried out with cell numbers and treatment being selected by the care provider (*e.g.*, physician). In some embodiments, the antigen-presenting cells and/or lymphocytes are administered in a pharmaceutically acceptable carrier. Suitable carriers may be growth medium in which the cells were grown, or any suitable buffering medium such as phosphate buffered saline. The cells may be administered alone or as an adjunct therapy in conjunction with other therapeutics.

In another aspect encompassed by the present invention, provided herein is a method for eliciting an immune response to a cell that expresses MAGEC2, the method comprising administering to the subject cells described herein expressing a binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof) in effective amounts sufficient to elicit the immune response. In some embodiments, provided herein is a method for treatment or prophylaxis of a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression), the method comprising administering to the subject an effective amount of the cells described herein expressing a binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof). In one embodiment, the cells are administered systemically, such as by injection. Alternately, one may administer locally rather than systemically, for example, via injection directly into tissue, such as in a depot or sustained release formulation.

In some embodiments, the cells described herein expressing a binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof) may be used as active compounds in immunomodulating compositions for prophylactic or therapeutic treatment of a disorder characterized by MAGEC2 expression (*e.g.*, a non-malignant disorder, a hyperproliferative disorder, or a relapse of a hyperproliferative disorder characterized by MAGEC2 expression). In some embodiments, MAGEC2-primed antigen-presenting cells

may be used for generating lymphocytes (*e.g.*, $CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes, and/or B lymphocytes), for further use in adoptive transfer to the subject with the cells described herein expressing a binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof).

In some embodiments, the cells described herein expressing a binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof), either alone or in combination with the lymphocytes, may be administered to a subject for eliciting an immune response, particularly for eliciting an immune response to cells are expressing MAGEC2.

As described above, single or multiple administrations of the cells described herein expressing a binding protein (*e.g.*, engineered TCR, CAR, or antigen-binding fragment thereof) cells, either alone or in combination with the lymphocytes, may be carried out with cell numbers and treatment being selected by the care provider *(e.g.,* physician). Similarly, the cells, either alone or in combination with lymphocytes, may be administered in a pharmaceutically acceptable carrier. Suitable carriers may be growth medium in which the cells were grown, or any suitable buffering medium such as phosphate buffered saline. Cells may be administered alone or as an adjunct therapy in conjunction with other therapeutics.

XI. Kits and devices

The present invention also encompasses kits and devices. For example, the kit or devie may comprise binding proteins, nucleic acids or vectors comprising sequences encoding binding proteins, host cells comprising nucleic acids or vectors and/or expressing the binding proteins as described herein, stable MHC-peptide complexes, adjuvants, detection reagents, and combinations thereof, packaged in a suitable container and may further comprise instructions for using such reagents. The kit may also contain other components, such as administration tools packaged in a separate container. The kit may be promoted, distributed, or sold as a unit for performing the methods encompassed by the present invention.

The disclosure is further illustrated by the following examples, which should not be construed as limiting.

EXAMPLES

Examples 1 and 2: Materials and Methods for Figures 1-3

(i) T cell engineering

CD8+ T cells (T Cells) were isolated from leukopaks using Miltenyi MultiMACS™ Cell24 Separator (Miltenyi Biotec) and the StraightFrom® Leukopak® Human CD8 MicroBead Kit following manufacturer's instructions (Miltenyi Biotec, cat. #130-117-019). Isolated CD8 T cells of >90% purity were resuspended at 10 x 10^6 cells/mL in CryoStor® CS10 (StemCell Technologies, cat. #07930, Cambridge, MA) and stored at -170C for subsequent experiments.

CD8 T cells were first thawed, resuspended in RPMI-1640 Medium (ATCC, cat. #30-2001) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS) (ThermoFisher, cat. #A3840002), 1X penicillin-streptomycin (ThermoFisher, cat. #15140122), 50 IU/mL recombinant human IL-2 (Peprotech, cat. #200-02), 5 ng/uL recombinant human IL-7 (R&D, cat. #207-IL), and 5 ng/uL recombinant human IL-15 (R&D, cat. #247-ILB), and rested overnight at 37C under 5% CO_2. The next day, the cells were activated with ImmunoCult™ human CD3/CD28 T cell activator (StemCell Technologies, cat. #10971) for 16 hrs and then transduced with individual TCRs packaged into lentivirus at 1 x 10^8- 1x 10^9 U/mL. 72 hours later, the residual virus was washed out and cells were pooled to create a library of TCR transduced T cells. Cells expressing an antibody tag were labeled with a biotin-labeled antibody and isolated using anti-biotin-conjugated microbeads (Miltenyi, cat. #130-090485) following the manufacturer's instructions. Isolated cells were expanded for seven days, resuspended in CryoStor® CS10 (StemCell Technologies, cat. #07930, Cambridge, MA) and frozen.

a. TCR selection with cytokine release and cytotoxicity assays.

Transduced TCRs were screened by measuring cytokine release when co-cultured with MAGEC2 expressing cell lines. Wild-type cancer cell lines were seeded at 6x10^4 cells in a 96-well flat bottom plate and rested for 16 hours at 37°C in 5% CO_2. On the following day, T cells were thawed, washed in complete RPMI-1640 medium, and added to the wells for an effector to target ratio of 1:1. The co-culture was incubated for 16 hours. Cells were then harvested by pipetting, transferred to V-bottom 96-well plates, and centrifuged at 2,000 rpm for 2 minutes. The supernatant was collected for Granzyme, IFNγ, TNFa, and IL-2 measurement using a human IFNγ 3rd generation multi-plex assay (Protein Simple). For flow cytometry analysis (Cytoflex S™ instrument, Beckman Coulter), cell pellets were

washed with FACS buffer (PBS, 0.5% BSA, 2 mM EDTA) and stained for activation-induced markers, including PE-conjugated anti-CD137 (Miltenyi), AF647-conjugated anti-CD69 (Biolegend), and BV421-conjugated anti-CD8 (BioLegend).

(i) For cancer cell culture and Incucyte® NucLight™ Red transduction

cancer cell lines were purchased from ATCC (Manassas, VA). . A101D, A2058, FTC133, and A2058 cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin and incubated at 37°C in 5% CO_2. Untransduced cancer cells were maintained in preparation for the T cell recognition assay, and 10^6 cells of each cell line were introduced with Incucyte® NucLight™ Red lentivirus reagent (Sartorius, Göttingen, Germany). Positively-labeled cells were selected using 1.5 ug/mL of puromycin over 72 hours.

(ii) Incucyte® cytotoxicity assay

Cancer cells selected for Incucyte® NucLight™ analysis were seeded into 96-well flat bottom plates (Corning, Flintshire, UK) at 10^4 cells. On the same day, T cells were thawed, washed in complete RPMI-1640, and allowed to rest overnight in medium with 50 U/mL IL-2 (PeproTech), 5 ng/mL IL-7, and 5 ng/mL IL-15 (R&D Systems). All cells were rested for 16 hours at 37°C in 5% CO_2. A two-fold serial dilution of the T cells was performed for the effector to target ratios, with the highest E:T at 10:1, and the lowest at 1:2. One image per whole well from two technical replicates was taken every 2 hours over a 72 hour time course. Imaging utilized a 4x objective lens and data were analyzed using the Incucyte® Basic Software. Red channel acquisition time was 400 ms, and its background was subtracted by enabling Top-Hat segmentation. Edge split was applied so that the software can recognize closely spaced cells as multiple objects, rather than one object, if the setting were absent.

Results of these experiments are shown in Figures 1-3.

Example 3: Further confirmatory characterization of TSC-201-B0702 action

The following representative Examples 3-8 further confirm the data and results presented in working examples 1-2 and continue to be based, in part, on the recognition that adoptive cell transfer with genetically engineered T cells holds great promise for treating solid tumors. Patients positive for particular HLA alleles of interest, such as HLA-B*07:02, are amenable to treatment with TCRs recognizing epitopes of a given target presented by such HLAs, such TSC-201-B0702.

For representative Examples 3-8, process-representative TSC-201-B0702 TCR-T cells (*e.g.*, helper ($CD4^+$, now $CD8^+/CD4^+$) and cytotoxic ($CD8^+$) T cells) were engineered by

transposon/transposase-mediated gene delivery of vector pNVVD187_TSC-201-B07_TCR-6_MSCV-TCR-6-CD8-EF1α-dnTGFbRII-DHFR as shown in FIG. 21A (unless otherwise indicated), to express (1) a recombinant TCR (*e.g.*, the recombinant TCR specific to the MAGE-C2-derived peptide RAREFMELL presented on HLA-B*07:02), (2) recombinant CD8α and CD8β co-receptors to maximize the efficacy of the therapeutic product, (3) a CD34-derived epitope tag fused on the N-terminus of CD8α to facilitate tracking of engineered cells *in vitro* and *in vivo*, (4) a mutated form of dihydrofolate reductase (DHFRdm) protein to facilitate enrichment of engineered cells during the manufacturing process, and (5) a dominant negative type II TGFβ receptor (DN-TGFβRII) to further address tumor microenvironment-mediated immune suppression. Such cells can be manufactured using known techniques and, for the present example, were generated through isolation of peripheral blood mononuclear cells (PBMC) from a fresh apheresis product, delivery of transposase mRNA and vector transposon npDNA by electroporation, T cell activation and culture, engineered cell enrichment via addition of methotrexate (MTX) in culture medium (selective growth advantage of engineered cells conferred by DHFRdm expression from transposon vector), cell washing to remove MTX, culture expansion, and culture wash, formulation, cryopreservation.

TSC-201-B0702 TCR-T cells were analyzed by flow cytometry to characterize the cell composition. The clinically representative TSC-201-B0702 TCR-T cells contain engineered (*i.e.*, $CD34^+$) helper ($CD4^+$, now $CD4^+/CD8^+$) and cytotoxic ($CD8^+$) T cells. The engineered helper and cytotoxic T cells express the recombinant TCR able to recognize the MAGE-C2-derived peptide RAREFMELL bound to HLA-B*07:02 as demonstrated by the binding of the relevant dextramer. Engineered TCR-T cells also express DN-TGFβRII.

Functional data were generated to characterize the mechanism of action of TSC-201-B0702. The data demonstrate that clinically representative TSC-201-B0702 TCR-T cells from at least 3 independent batches (subjects) react to the MAGE-C2-derived peptide RAREFMELL in a dose-dependent manner when presented by target cells on the HLA-B*07:02 MHC in peptide pulse experiments. Three representative, independent batches of TCR-T cells generated from different donors were tested for their reactivity against their cognate peptide/MHC. As described further below, TSC-201-B0702 TCR-T cells were co-cultured with peptide pulsed T2 cells. T2 cells were pulsed with a titration of the MAGE-C2-derived peptide RAREFMELL and TCR reactivity was determined by the peptide dose-dependent cytotoxicity against the T2 cells. This study confirmed that the TSC-201-B0702

specifically reacts to cells presenting the MAGE-C2-derived peptide RAREFMELL presented on HLA-B*07:02.

When encountering a target cell naturally expressing MAGE-C2 and HLA-B*07:02, TSC-201-B0702 TCR-T cells secrete pro-inflammatory cytokines and granzyme B and mount a proliferative response detected both in the engineered cytotoxic and helper T cell subpopulations. The TCR-T cells eventually kill these target cells naturally presenting the MAGE-C2-derived peptide RAREFMELL on HLA-B*07:02. Here, a panel of target cancer cell lines naturally expressing MAGE-C2 and HLA-B*07:02 was used in co-culture assays with the 4 independent batches of TSC-201-B0702 TCR-T cells to evaluate the biological outcomes regarding TSC-201-B0702 TCR engagement. Untransfected (UTF) control T cells obtained from matching donors were used as negative controls. Untransfected (UTF) control T cells were also produced from the isolated PBMCs used to generate each batch of TSC-201-B0702 process-representative material tested. The UTF cells did not undergo electroporation or MTX based enrichment, but were similarly activated and cultured as the TSC-201-B0702 TCR-T cells. Target cells positive for HLA-B*07:02 expression but negative for MAGE-C2 were used as additional negative controls. Multiple readouts were used to evaluate the functional engagement of the TCR-T cells, including: (1) secretion of pro-inflammatory cytokines (IFN-γ, TNF-α and IL-2) and granzyme B in the co-culture supernatant was evaludated 24 hours post co-culture; (2) proliferation of the engineered T cells (both $CD4^+$ and $CD4^-$) was assessed after ~4 days post co-culture; and (3) selective cytotoxic activity was examined over 3 days of co-culture.

In addition, TSC-201-B0702 TCR-T cells displayed reduced phospho-SMAD2 induction after exposure to TGFβ, demonstrating that the expression of dominant negative TGFβ type II receptor in TSC-201-B0702 TCR-T cells generates resistance to TGFβ signaling in TSC-201-B0702 TCR-T cells. The ability of the DN-TGFβRII-expressing TCR-T cells to resist TGFβ signaling was tested by demonstrating reduced SMAD2 phosphorylation after stimulation of TCR-T cells with physiological levels of TGFβ-1.

Materials and methods for preparing effector T cells and target cells, as well as seting up co-cultures, are described herein. T cells were thawed in a 37°C water bath and washed once with cytokine-free T cell medium to remove cryopreservation reagents prior to being resuspended in complete T cell medium. T cells were then seeded in a G-REX® 6-well plate at a density of 1-2E6 live cells/mL and allowed to recover in a humidified incubator at 37°C and 5% CO2 for 16-24 hours prior to co-culturing. The next day, T cells were harvested, washed, and resuspended in cytokine-free T cell medium at an assay-dependent cell density.

Similarly, target cells (Table 5) were prepared. For example, cancer cell lines were thawed in a 37°C water bath and washed once with their respective cell culture medium (Table 5) to remove cryopreservation reagents. Cells were subsequently resuspended in their respective cell culture medium and cultured following standard procedures in 75 cm^2, 175 cm^2 or 225 cm^2 flasks (adherent target cells) or G-REX® wells (suspension cells) in a humidified incubator at 37°C and 5% CO_2. The cancer cell lines were maintained in culture at least one passage, and no longer than 3-4 weeks prior to the initiation of the co-culture with T cells. Target cells, except for COLO-783 cells, which were expanded for ~ 2 months prior to the initiation of the co-culture assays due to the slow growth of this cell line.

The following discussion further details certain experiments and results introduced above.

a. Flow cytometric analysis of process-representative TSC-201-B0702 TCR-T cells.

First, the cellular composition of TSC-201-B0702 TCR-T cells was examined by flow cytometry. Process-representative test articles and their untransfected (UTF) donor matched-controls were thawed as described as described above. After overnight recovery, all T cells were washed, labeled with antibodies from the "Dextramer Panel" (Table 5) and acquired. Unstained cells, single stain controls and FMO controls were also prepared.

Table 5. Cancer cell, media, flow cytometry "Dextramer Panel", and T cell proliferation detection reagents, and appendix reagents, respectively

Cancer cell reagents

Cancer cell line name	Indication	Source	Catalogue number	MAGE-C2 *(TPM)*	HLA-B*07:02 *(TPM)*
COLO-783	Melanoma	DSMZ*	ACC 257	+ *(265)*	+ *(424)*
A101D	Melanoma	ATCC	CRL-7898	+ *(209)*	+ *(303)*
FTC-133	Follicular thyroid carcinoma	Sigma Aldrich	94060901	+ *(128)*	+ *(268)*
A2058	Melanoma	ATCC	CRL-3601	- *(4.53)*	+ *(45.3)*
T2	Lymphoblast	ATCC	CRL-1992	*NA*	*NA*

Media reagents

Media	Components	V stock (mL)	Catalog Number	Notes
Cytokine-free T cell medium	X-VIVO 15	1000	Lonza #04-418Q	Expires 30 days after preparation
	Human Serum (Heat-Inactivated) 5%	50	Gemini Bio #100-512	
	Penicillin/Streptomycin 1%	10	Thermofisher Scientific #15149-122	
	GlutaMAX (100x) 1x	10	Thermofisher Scientific #35050061	
	Total	**1070**	n/a	
Complete T cell medium	**Cytokine-free** T cell media	200	n/a	Do not store; prepare and use fresh
	Human IL-2 (5 µg/mL)	1	Sigma-Aldrich #11147528001	
	Human IL-7 (10 µg/mL)	0.1	R&D Systems #207-IL-025	
	Total	**201.1**	n/a	
RPMI-based medium (COLO783)	RPMI 1640	500	ATCC 30-2001	Expires 30 days after preparation
	Fetal Bovine Serum 10%	50	Thermofisher Scientific #A3840001	
	Penicillin/Streptomycin 1%	5	Thermofisher Scientific #15149-122	
	Total	**555**	NA	
DMEM-based medium (A101D, A2058)	DMEM	500	Thermofisher Scientific #11965092	Expires 30 days after preparation
	Fetal Bovine Serum 10%	50	Thermofisher Scientific #A3840001	
	Penicillin/Streptomycin 1%	5	Thermofisher Scientific #15149-122	
	Total	**555**		
DMEM/F12-based medium (FTC-133)	DMEM/F12	500	ATCC #30-2006	Expires 30 days after preparation
	Fetal Bovine Serum 10%	50	Thermofisher Scientific #A3840001	
	Penicillin/Streptomycin 1%	5	Thermofisher Scientific #15149-122	
	Total	**555**	NA	
IMDM-based medium (T2)	**IMDM**	**500**	ATCC #30-2005	Expires 30 days after preparation
	Fetal Bovine Serum 20%	**100**	Thermofisher Scientific #A3840001	
	Penicillin/Streptomycine 1%	**5**	Thermofisher Scientific #15149-122	
	Total	**605**	NA	

Flow cytometry "Dextramer panel" reagents

Antibody	Clone	Vendor	Fluorochrome	Catalog Number
near-IR fluorescent reactive dye	N/A	Thermofisher Scientific	Near-IR (APC-Cy7)	L34976A
CD34	QBEND/10	Thermofisher Scientific	Biotinylated	MA5-16924
Streptavidin	N/A	BioLegend	AF488	405235
CD4	RPA-T4	BioLegend	PE-Cy7	300512
CD8b	2ST8.5H7	BD Bioscience	BV421	568373
TGFβRII	W17055	BioLegend	PE	399704
Dextramer	N/A	Immudex	APC	WH06649 AP 500

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T cell proliferation detection reagents

Reagent	Clone	Vendor	Fluorochrome	Catalog Number
Far Red Fixable Live-Dead Dye	N/A	Thermofisher Scientific	APC equivalent	L34974
CD34	QBEND/10	R&D Systems	Alexa Fluor 488	FAB7227G
TCR α/β	IP26	Biolegend	PE-Cy7	306720
CD4	OKT4	Biolegend	APC-Cy7	317418
CD8	HIT8α	Biolegend	PerCP-Cy5.5	300924
CellTrace Violet (CTV)	NA	Thermofisher Scientific	PB450	C34557

Phospho-Flow reagents

Antibody	Clone	Vendor	Fluorochrome	Catalog Number
Human CD34	QBEND10	R&D Systems	Alexa Fluor® 488	FAB7227G
Phospho-SMAD2 (Ser465/Ser467) Rabbit mAb	E8F3R	Cell Signaling Technology	Alexa Fluor® 647 Conjugate	68550S
SMAD2 XP® Rabbit mAb	D43B4	Cell Signaling Technology	Alexa Fluor® 647 Conjugate	67617S

Reagent	Vendor	Catalog Number
Human Transforming Growth Factor β1 (hTGF-β1) (25ug)	R&D Systems	7754-BH-025/CF
Reconstitution Buffer 3 (HCl)	R&D Systems	RB03
BD Phosflow Fix Buffer I	BD	557870
Methanol (99.8%) (125mL)	Cell Signaling Technology	13604S
Flow Cytometry Antibody Dilution Buffer (125mL)	Cell Signaling Technology	13616S
LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit, for 405 nm excitation	Thermofisher Scientific	L34959

Appendix reagents

Antibody	Clone	Vendor	Fluorochrome	Catalog Number
near-IR fluorescent reactive dye	N/A	Thermofisher Scientific	Near-IR (APC-Cy7)	L34976A
Live/DeadTM Far Red Dye	N/A	Thermofisher scientific	Far IR	L34973
CD34	QBEND/10	Thermofisher Scientific	Biotinylated	MA5-16924
Streptavidin	N/A	BioLegend	Alexa488	40235
CD4	RPA-T4	BioLegend	PE-Cy7	300538
CD4	Okt4	BioLegend	APC-Cy7	317418
CD8b	2ST8.5H7	BioLegend	BV421	742390
CD8	Hit8a	Biolegend	Percp-Cy5	300924
TGFβRII	W17055	BioLegend	PE	399704
MAGE-C2/HLA-B*07:02 Dextramer	MAGE-C2 (RAREFMELL)	IMMUDEX	APC	Custom (HLA-B*07:02/ RAREFMELL)
TCRαβ	IP26	BioLegend	PE-Cy7	306720
Count BrightTM Absolute counting beads	N/A	Thermofisher scientific	N/A	C36950
UltraComp ebeadsTM plus	N/A	Thermofisher scientific	N/A	01-33333-42
Human CD34	QBEND10	R&D Systems	Alexa Fluor® 488	FAB7227G

Phospho-SMAD2 (Ser465/Ser467) Rabbit mAb	E8F3R	CST	Alexa Fluor® 647 Conjugate	68550S
SMAD2 XP® Rabbit mAb	D43B4	CST	Alexa Fluor® 647 Conjugate	67617S
LIVE/DEAD™ Fixable Yellow Dead Cell Stain Kit, for 405 nm excitation	n/a	Invitrogen	Yellow (violet610)	L34959

Peptide	Peptide Sequence	Gross Weight	Purity	Vendor	Catalog Number
MAGE-C2	RAREFMELL	2.0mg	97.9%	GenScript	Sc1208

Media	Vendor	Catalog Number
IMDM	ATCC	30-2005
XVIVO 15	Lonza	04-418Q
DMEM +D-Glucose +L-Glutamine	ATCC	11965-092
DMEM/F12	ATCC	30-2006
RPMI1640	ATCC	30-2001
Human Serum (Heat-Inactivated)	MP CELLect 100ml	2930949
Human Serum (Heat-Inactivated)	GeminiBio	100-512
Penicilline-Streptomycin	Thermofisher Scientific	15149-122
100x Glutamax	Thermofisher Scientific	35050-061
FBS, heat-inactivated	Thermofisher Scientific	A3840001

Reagent	Vendor	Catalog Number
DMSO	Sigma Life Science	D2650-100ML
DPBS/Modified	Gibco	14190-144
ViaStain AOPI Staining Solution	Nexcelom Bioscience	CS2-0106-25mL
70% Denatured Ethanol	Decon Laboratories inc	8601
Poly-L-Ornithine Solution, 0.01%	Millipore-Sigma	P4957
recombinant human IL2	Sigma-Aldrich	11147528001
recombinant human IL7	RD Systems	207-IL/CF
reconstitution buffer 2 (0.1% BSA in PBS)	RD Systems	RB02
EasysepTM buffer	Stem Cell Technologies	20144
anhydrous DMSO	Thermofisher Scientific	D12345
CellTraceTM Violet proliferation kit	Thermofisher Scientific	C34557
BD CytofixTM Fixation Buffer	BD Bioscience	554655
Human Transforming Growth Factor β1 (hTGF-β1) (25ug)	RD Systems	7754-BH-025/CF
reconstitution buffer 4 (HCl-BSA)	RD Systems	RB04
Reconstitution Buffer 3 (HCL)	R&D Systems	RB03
BD Phosflow Fix Buffer I	BD	557870
Methanol (99.8%) (125mL)	CST	13604S
Flow Cytometry Antibody Dilution Buffer (125mL)	CST	13616S

Data acquisition was performed on a Cytoflex S. Compensation was performed automatically with CytExpert software. Flow cytometry analysis was performed with FlowJo v10.8.1. The statistics were exported to Excel 2010 and analyzed.

The following description provides the cell gating strategy. Briefly, cells were gated from the FSC versus SSC dot plot and singlets distinguished from the aggregates using FSC-Area versus FSC-Height plot. Viable cells were identified using the Near-Infrared Live-Dead versus FSC-Area plot. Subpopulations were gated from the viable cells and evaluated, including, $CD4^+$ (Helper T cells), $CD4^+/CD8^+$ (Engineered Helper T cells), $CD4^-/CD8^+$ (Cytotoxic T cells), and $TGF\beta RII^+/CD34^+$ (engineered TCR-T cells expressing DN-TGFβRII). Cells identified as $CD34^-$ or $CD34^+$ were further evaluated regarding TGFβRII expression. The mean fluorescent intensity (MFI) was measured for $CD34^-$/TFGβRII and $CD34^+$/TFGβRII.

Flow cytometric analysis confirmed that TSC-201-B0702 TCR-T cells from four different batches contained engineered helper cells ($CD4^+$, now $CD4^+/CD8^+$), residual non-engineered helper T cells ($CD4^+/CD8^-$), and cytotoxic ($CD8^+$) T cells. The percentage of non-engineered $CD4^+/CD8^-$ helper T cells ranged from 8.51% to 11.70%. Engineered helper T cells expressed the exogenous CD8αβ co-receptor and were characterized as $CD4^+/CD8^+$. The batches of TSC-201-B0702 TCR-T cell material presented between 21.85% and 33.05% of these engineered helper T cells and the percentage of cytotoxic T cells ($CD4^-/CD8^+$) ranged from 54.20% to 67.90%.

The engineered T cells were confirmed to express the recombinant TCR specific to the MAGE-C2-derived peptide RAREFMELL bound to HLA-B*07:02, as revealed by dextramer binding, as well as the CD34 epitope. No dextramer positive or CD34 positive population was detected in untransfected (UTF) T cells from matched donors, illustrating the highly specific nature of the staining. For the four batches of TSC-201-B0702 TCR-T cells tested, the percentage of the $dextramer^+/CD34^+$ population ranged from 43.55% to 62.70%.

Both the engineered $CD4^-$ and $CD4^+$ T cells were able to bind the dextramer, confirming that functional TCRs were found on the surface of both the helper and cytotoxic T cells. The percentage of $Dextramer^+/CD4^-$ populations ranged from 26.90% to 43.25%, and the percentage of $Dextramer^+/CD4^+$ ranged from 8.98% to 29.60%.

Engineered T cells making up TSC-201-B0702 material expressed the DN-TGFβRII, as evidenced by a marked increase in the $TGF\beta RII^+$ signal in the $CD34^+$ population when compared to non-engineered $CD34^-$ cells.

b. pMHC dose-dependent function of the recombinant TCR expressed by TSC-201-B0702 TCR-T cells

Figures 4A and 4B show reactivity of TSC-201-B0702 TCR-T cells from three independent donors against their cognate peptide/MHC as determined using a peptide pulse assay. Cancer cell line T2 was purchased from ATCC and cultured per the vendor's recommendations. T2 lymphoblast cells do not naturally express HLA-B*07:02 allele of the MHCI. T2 cells were engineered with IncuCyte® NucLight Lentivirus Red Reagents to enable imaging of cells. The naturally expressed MHCI was knocked out so that the NucLight™ Red positive T2 cells could then be transduced by Lentivirus Reagent to express HLA-B*07:02 (T2-B07). NucLight™ Red-transduced T2-B07 target cells were peptide-pulsed with a titration of the MAGE-C2 peptide RAREFMELL and co-cultured with TSC-201-B0702 TCR-T cells. TCR reactivity was assessed by measuring the growth of target NucLight™ Red-transduced T2-B07 cells.

T2-B07 cells were harvested and washed with serum free media and resuspended to the desired cell concentration of 3E5 cells/mL. One mL of the cell suspension was then transferred to separate vessels to be pulsed with varying concentrations of the MAGE-C2 peptide, RAREFMELL. Peptide titrations were prepared in serum free IMDM media, ranging from 10,000,000 pg/mL to 0.01pg/mL. T2-B07 cells were resuspended in 1 mL of diluted peptide and incubated in a humidified incubator at 37°C and 5% CO_2 for 45 minutes to an hour. Peptide-pulsed target cells were then washed with serum-supplemented IMDM media and cell density for each peptide pulsed condition was adjusted to 1E5 live cells/mL.

On the day of the co-culture, T cells were harvested, washed with cytokine-free T cell media, and resuspended in cytokine-free media to the desired cell density of 5E5 live cell/mL. One hundred µL of each peptide pulsed T2-B07 cells was added to its respective well, resulting in 1E4 viable cells plated per well. Prepared TCR-T cells were then resuspended and 100µL (5E4 viable cells total) were added on top of the pulsed target cells for a final E:T of 5:1. For target cells alone condition, 100 µL of cytokine free T cell media was added on top of the target cells. Co-cultures were allowed to settle for 15 minutes in a humidified incubator at 37°C and 5% CO_2 prior to the initiation of acquisition.

Samples were acquired in the IncuCyte® S3 Live-Cell Analysis system (Sartorius, Goettingen, Germany). IncuCyte® acquired data were analyzed using IncuCyte® S3 Live-Cell Analysis system software GUI Version 2021A (Sartorius, Goettingen, Germany). A customized analysis definition was created to quantify the area occupied by red objects (*i.e.*, NucLight™ Red-positive T2 cells) over time. Target cell growth is measured in Red

Channel Areas Per Image (4 per well) and normalized to 0d0h0m.The raw data was exported and graphed in GraphPad Prism (v9.2.0) Time in hours was plotted on the x-axis and target cell growth plotted as a percentage on the Y-axis. The area under the curve (AUC) of the T2 cell growth for each peptide dose was calculated using GraphPad Prism software. The AUCs were plotted (y-axis) as a function of peptide dose (x-axis) and a nonlinear regression curve (using the equation Sigmoidal, 4PL, X is concentration) was used to graph the dose-dependent cytotoxicity of the test articles.

The cytotoxic activity of three batches of TSC-201-B0702 TCR-T cells against peptide-pulsed T2-B07 cells was measured over time in an IncuCyte® S3 imager by quantifying the relative growth of the NucLight™ Red-labeled T2-B07 cells (Figure 4A). The normalized area under the curve (AUC) for the resulting growth of T2-B07 cells over 72 hours of co-culture with 2 batches of TCR-T cells as a function of the peptide concentration were plotted (Figure 4B). Unpulsed T2-B07 cells do not present endogenous peptide on their surface (Steinle & Schendel (1994) *Tissue Antigens* 44:268–270) and served as a negative control. For the two batches of process-representative material tested in the cytotoxicity assay, no cytotoxicity was observed when the TSC-201-B0702 TCR-T cells were co-cultured with unpulsed T2 negative controls (see 0 ng/mL in Figures 4A and 4B).

T2 cells loaded with MAGE-C2 peptide (RAREFMELL) served as target cells presenting exogeneous peptide on HLA-B*07:02. To assess the peptide dose-dependent function of TSC-201-B0702 TCR-T cells, T2-B07 cells were exogenously loaded with a titration of RAREFMELL peptide and co-cultured with two batches of process-representative TSC-201-B0702 TCR-T cells at an effector to target ratio (E:T) of 5:1.

As shown in Figures 4A and 4B, both batches of TSC-201-B0702 material tested displayed a similar dose-dependent cytotoxic activity toward T2-B07 cells pulsed with the MAGEC2-derived peptide RAREFMELL. Across the 2 batches of TSC-201-B0702 material tested cytotoxicity was observed at 5 ng/mL and saturated at ~10 ng/mL. Altogether, these data confirm that TSC-201-B0702 TCR-T cells are highly specific and efficiently react to the MAGE-C2-derived peptide RAREFMELL presented on HLA-B*07:02.

c. TSC-201-B0702 TCR-T cells exhibit target-dependent cytokine and Granzyme B production

Target-dependent induction of Granzyme B secretion as well as secretion of the pro-inflammatory cytokines IFN-γ, IL-2 and TNF-α was assessed after ~24 hours co-culture of four batches of TSC-201-B0702 TCR-T cells with a panel of cancer cell lines endogenously

expressing MAGE-C2 and HLA-B*07:02 (Table 5). A2058 cells, which are positive for HLA-B*07:02 but negative for MAGE-C2, as well as donor-matched untransfected (UTF) control T cells were included as negative controls in the experiment. A 'T cell only' condition wherein TSC-201-B0702 TCR-T cells or UTF control T cells were cultured in the absence of any target cells was used to establish baseline levels of Granzyme B, IFN-γ, IL-2 and TNF-α in the assay.

Specifically, the day before initiation of the co-culture, target cells were plated in 100 µL target cell medium (Table 5) in round bottom 96 well plates. Target cells were seeded at 1E5 live cells/well (COLO783, FTC-133) or 5E4 live cells/well (A101D, A2058) to achieve a cell density of 1E5 cells per well on the day of the initiation of the co-culture. To initiate the co-culture, TSC-201-B0702 TCR-T cells and UTF controls were diluted to 1E6 live cells/mL in cytokine-free T cell medium and 100 µL of the cell solution (corresponding to 1E5 live T cells) were added to the target cells, resulting in an E:T of 1:1. As a control, both effector alone and target alone wells were prepared; these controls received 100 µL of RPMI1640 10% FBS 1% PS or 100 µL of cytokine-free T cell medium, respectively.

After 24 hours co-culture at 37°C 5% CO_2, supernatants were collected and stored at -80°C. Subsequently, IFN-γ, TNF-α, IL-2 and Granzyme B secreted into the supernatant was quantified on an automated 4-plex ELISA platform (ELLA from Protein Simple) according to the manufacturer's instructions. Simplex Explorer software was used to run samples on an automated ELISA platform (ELLA). Raw data were exported as excel file and the concentration was calculated by multiplying the reported cytokine concentration with the dilution factor. Data were plotted in Graphpad Prism (v10).

When TSC-201-B0702 TCR-T cells were co-cultured with the negative control cell line A2058, cytokine secretion of IFN-γ, IL-2, and TNF-α were similar to the baseline secretion observed in the 'T cell only' condition (see Figures 5A-5C).

Upon co-culture of TSC-201-B0702 TCR-T cells with COLO-783 and FTC-133, which express MAGE-C2 and HLA-B*07:02, a robust induction of IFN-γ, TNF-α and IL-2 secretion was observed for all four batches of TSC-201-B0702 TCR-T cells (see Figures 5A-5C). When compared to 'T cell only' condition, TSC-201-B0702 TCR-T cells showed a ~47-729-fold induction of IFN-γ, ~8-166-fold-induction of IL-2 and ~23-352-fold induction of TNF-α when co-cultured with COLO-783 (dark green bars) and FTC-133 (light green bars) cell lines. TSC-201-B0702 TCR-T cells secreted less cytokines upon co-culture with the A101D cell line than upon co-culture with the other two MAGE-C2 positive cell lines (Figures 5A-5C). For the A101D cell line, IFN-γ induction ranged from 3-to 19-fold, IL-2

induction from ~1.4-2.6-fold and TNF-α induction from ~8-19-fold. The levels of cytokine secretion from UTF control T cells remained much lower than when TSC-201-B0702 TCR-T cells were used in the co-cultures, confirming the lack of significant activation of the T cells in the absence of the therapeutic TCR (Figures 5E-5G).

Upon co-culture with MAGE-C2 positive and HLA-B*07:02-positive target cell lines, an increase in Granzyme B secretion was observed with all four batches of TSC-201-B0702 TCR-T cells (Figure 5D). In comparison to the 'T cells only' condition, co-cultures with A101D, FTC-133, and COLO-783 induced a ~4-10-fold, ~11-15-fold and ~11-41-fold increase, respectively, of Granzyme B secretion in TSC-201-B0702 TCR-T cells. The target cell lines induced little or no Granzyme B secretion in UTF control T cells, demonstrating lack of activation of donor T cells in the absence of the therapeutic TCR (Figure 5H). Although the MAGE-C2 negative cell line A2058 stimulated Granzyme B secretion in one of the four batches of TSC-201-B0702 TCR-T cells (PD356F, see Figure 5D), TSC-201-B0702 TCR-T cells from batch PD356 were not activated by A2058 cells based on oberservations that TSC-201-B0702 TCR-T cells from batch PD356F did not produce IL-2, IFN-γ or TNF-α when co-cultured with the A2058 target cells, failed to proliferate in response to A2058 target cells (Figure 6A), and did not present any cytotoxic function against the A2058 cell line (Figures 7A and 7B). Taken together, these data show that TSC-201-B0702 TCR-T cells do not react to HLA-B*07:02-positive target cells that do not express the target protein MAGE-C2.

Taken together, these data demonstrate that TSC-201-B0702 TCR-T cells operate in a target-dependent manner, reacting to target cell lines endogenously expressing MAGE-C2 and HLA-B*07:02 by secreting pro-inflammatory cytokines and Granzyme B.

d. TSC-201-B0702 TCR-T cells proliferate in a target-dependent manner

Target-dependent proliferation of the engineered T cells (both $CD4^+$ and $CD4^-$) was also assessed. Briefly, target cells were plated one day prior to initiating the co-culture in 100 µL of target cell medium (Table 5) in 96 well flat bottom plates. Target cells were seeded at 1E5 live cells per well (COLO783, FTC-133) or 5E4 live cells per well (A101D, A2058) to achieve cell density of 1E5 cells per well on the day of the initiation of the co-culture.

Process-representative TSC-201-B0702 TCR-T cells and their donor-matched UTF controls were thawed and allowed to recover overnight as described above. To eliminate baseline proliferation induced by the T cell cytokines IL-2 and IL-7, effectors were washed once with cytokine-free T cell media, re-seeded in 6-well G-Rex® plates at a concentration of

1E6-2E6 live cells/mL and incubated for 20-24 hours in cytokine-free T cell medium before initiating the co-culture. On the day of the inititation of the co-culture, effectors were stained with cell trace violet (CTV) dye as follows: effectors were washed once with EasySep buffer and stained for 7 minutes at room temperature with CTV dye (reconstituted according to manufacturer's instructions and diluted 1:2000 in EasySep buffer). After washing T cells twice with cytokine-free T cell media, the T cell concentration was adjusted to 1E6 live cells/mL in cytokine-free T cell medium, and 100 µL (corresponding to 1E5 live T cells) were added to the target cells to achieve an E:T of 1:1. As a control, both effector alone and target alone wells were prepared; these controls received 100 µL of cytokine-free T cell medium or 100 µL of RPMI1640 10% FBS 1% PS, respectively. To ensure that negative control wells (i.e., T cells co-cultured with the MAGE-C2 negative target cell line A2058, and T cell alone controls) contained enough T cells for evaluation of T cell proliferation, 12 replicates were set up for each control condition, and were pooled at the end of the co-culture into 3 replicates prior to staining. To minimize evaporation, plates were covered with BreathEasy membrane. Target and effectors were then co-cultured for 3.5 days at 37°C 5% CO2 in a humidified incubator. At the end of the co-culture, effectors were transferred to a v-bottom plate and stained with the staining reagents listed in Table 5, followed by fixation with Cytofix (BD Bioscience). After washing out the fixative, samples were resuspended in 100 µL EasySep buffer, and 10 µL of Absolute CountBright counting beads (Thermofisher Scientific) were added to the wells immediately before acquisition. Data acquisition was performed on a Cytoflex S (Beckman Coulter) using CytExpert Software. Data analysis was performed with FlowJo v10.8.1.

The following provides the cell gating strategy used. Briefly, for transfected T cells, the number of proliferating $TCR\alpha\beta^+CD34^+$ (total transfected T cells), proliferating $TCR\alpha\beta^+CD34^+CD4^+CD8^+$ (transfected helper T cells) and proliferating $TCR\alpha\beta^+CD34^+CD4^-CD8^+$ T cells (transfected cytotoxic T cells) was determined. For untransfected control T cells, the number of proliferating $TCR\alpha\beta^+$T cells (total T cells), proliferating $TCR\alpha\beta^+CD4^+$ (helper T cells) and proliferating $TCR\alpha\beta^+CD8^+$ (cytotoxic T cells) was determined. Raw data were exported as excel files, and the number of proliferating cells was normalized to the bead count. Normalized cell counts were then graphed in GraphPad Prism (v10).

Figures 6A-6B show results of evaluation of target-dependent induction of proliferation. The panel of cancer cell lines used to evaluate target-dependent induction of cytokine and granzyme B secretion was also used to assess target-dependent induction of T cell proliferation. The four independent batches of process-representative TSC-201-B0702

TCR-T cells and UTF control T cells were labeled with CTV dye and were cultured alone, or co-cultured with cancer cells for ~3.5 days at an ~E:T of 1:1. Subsequently, proliferation was evaluated as described in above.

When cultured in the absence of target cells ('T cell only') or in the presence of the negative control cell line A2058 (which expresses HLA-B*07:02 but is MAGE-C2 negative), TSC-201-B0702 TCR-T cells did not proliferate (see Figure 6A). These data demonstrate a lack of reactivity of TSC-201-B0702 TCR-T cells to HLA-B*07:02-positive target cells in the absence of MAGE-C2 protein. On the other hand, co-culture of TSC-201-B0702 TCR-T cells with COLO-783 and FTC-133, which express MAGE-C2 and HLA-B*B07:02, induced a robust proliferation of the TCR-T cells across all four batches of TSC-201-B0702: a 13-25-fold and 23-33-fold increase in the number of dividing engineered ($CD34^+$) T cells was observed in co-cultures with FTC-133 and COLO-783, respectively (Figure 6A). Both helper and cytotoxic TCR-T cells mounted a proliferative response upon co-culture with these MAGE-C2-positive, HLA-B*07:02-positive target cells. A101D stimulated weaker proliferative responses in TSC-201-B0702 TCR-T cells than the FTC-133 and COLO-783 cell line. Compared to the T cell alone control, the number of dividing T cells was only ~ 3-fold increased (Figure 6A). None of the target cell lines induced proliferation in UTF control T cells (Figure 6B), demonstrating once more that the T cell activation observed in the TSC-201-B0702 TCR-T cells is dependent on expression of the therapeutic TCR.

e. TSC-201-B0702 TCR-T cells exhibit selective and potent cytotoxic function

To test the target-dependent cytotoxic activity of TSC-201-B0702 TCR-T cells, four independent batches of process-representative TSC-201-B0702 TCR-T cells and donor matched UTF control T cells were used in an IncuCyte®-based cytotoxicity assay. The effector T cells were serially diluted and co-cultured with a fixed number of target cells to test different effector to target ratios (E:T). Untransfected (UTF) control T cells from matching donors were similarly tested as negative controls. The target cell lines tested corresponded to the panel also used to test induction of cytokine secretion and proliferation and comprised MAGE-C2-positive, HLA-B*07:02-positive cell lines (*i.e.*, COLO-783, FTC-133, and A101D) as well as a negative control target cell line (*i.e.*, A2058, which is HLA-B*07:02 positive, but negative for MAGE-C2). The target cells were engineered to express NucLight™ Red, a fluorescent protein to enable the tracking and quantification of cell growth over time in the IncuCyte® imager.

For the Incucyte®-based cytotoxicity assay, NucLight™ Red-transduced target cells were plated the day before initiating the co-culture in 100 µL target cell medium (Table 5) in 96 well flat bottom plates. Target cells were seeded at 1E4 live cells per well (COLO783, FTC-133) or 5E3 live cells per well (A101D, A2058) to achieve a cell density of ~1E4 cells per well on the day of the initiation of the co-culture.

Process-representative test articles and their donor-matched UTF controls were thawed and rested overnight as described above. Serial dilutions of effectors, TSC-201-B0702 TCR-T cells, and the donor-matched UTF T cells were prepared in cytokine-free T cell media to obtain the plating concentration, and 100 µL of effector T cells were added to the target cells, resulting in an effector to target ratio (E:T) ranging from 5:1 to 0.6:1. For the target cell only condition, 100 µL of cytokine-free T cell media was added to the target cells. Plates were sealed with BreathEasy® membrane to limit evaporation of media and were placed in a humidified incubator to equilibrate to 37°C for 10-15 minutes Subsequently, a Kimwipe was used to wipe off condensation of the bottom of the plates, plates were placed into the Incucyte Live-cell analysis instrument (Sartorius) and acquisition was started. Samples were acquired on an Incucyte® system as described above. Raw data were exported as excel sheet and the target cell growth normalized to the 0 hour time point was plotted in Graphpad Prism (v10). The area under the curve (AUC) was calculated for the growth curves obtained with target cells co-cultured with TSC-201-B0702 TCR-T cells and UTF control cells at an E:T of 2.5:1, and the AUC of growth curves of target cells co-cultured with TSC-201-B0702 TCR-T cells was normalized to the growth curves of target cells co-cultured with the corresponding UTF control.

Representative growth curves of target cells co-cultured for 3 days with TSC-201-B0702 TCR-T cells from batch PD354 and donor-matched UTF control cells are shown in Figure 7A, and the area under the curve (AUC) for growth curves obtained with TSC-201-B0702 TCR-T cells from all four batches at an E:T of 2.5:1, normalized to the AUC of the growth curves obtained with the corresponding UTF control TCR-T cells, are shown in Figure 7B.

In contrast to UTF control T cells, TSC-201-B0702 TCR-T cells displayed a dose-dependent and selective killing response, leading to inhibition of the target cell growth upon co-culture with the MAGE-C2-positive and HLA-B*07:02 positive cell lines COLO-783, FTC-133 and A101D (Figures 7A and 7B). As expected, little to no growth inhibition was

observed when TSC-201-B0702 TCR-T cells were co-cultured with the HLA-B*07:02-positive and MAGE-C2-negative cell line A2058.

In contrast, the cytotoxic response of TSC-201-B0702 TCR-T cells towards the cell line A101D was less pronounced and only evident at higher E:Ts. However, all four batches of TSC-201-B0702 were able to kill and restrict growth of A101D at E:Ts of 5:1 and 2.5:1 (Figures 7A and 7B).

Taken together, these data demonstrate that TSC-201-B0702 TCR-T cells can mount a potent and selective cytotoxic response towards MAGE-C2-positive target cell lines derived from HLA-B*07:02 tumor.

f. TSC-201-B0702 TCR-T cells exhibit resistance to TGFbeta signaling

Figure 8 shows the ability of the engineered TSC-201-B0702 TCR-T cells to resist TGFβ-mediated inhibition of T cell function. Specifically, a flow-cytometry-based measurement of the stimulation of SMAD2 phosphorylation was implemented. The induction of the phosphorylation of the transcription factor SMAD2 is part of the canonical TGFβ signal transduction cascade (Vander *et al.* (2018) *Cell Signal.* 52:112-120); phospho-SMAD2 quantification can be therefore used as read-out of TGFβ signaling.

Bridging studies were conducted to verify that reduced phospho-SMAD2 induction upon stimulation with physiological levels of TGFβ correlates with reduced TGFβ-mediated inhibition in cell-based functional assays. All data confirmed across multiple TCR-T cell products that the expression of DN-TGFβRII reduces the ability of the TCR-T cells to phosphorylate SMAD2 protein when exposed to TGFβ, and that reduced phospho-SMAD2 induction correlates with the ability of the TCR-T cells to maintain their target-dependent reactivity.

Following these bridging studies, flow cytometric analysis of the phosphorylation of SMAD2 in the presence and absence of TGFβ was used to evaluate the ability of process-representative TSC-201-B0702 to resist TGFβ signaling. In this assay, process-similar donor matched UTF control cells—which do not express DN-TGFβRII and in which TGFβ signaling is therefore intact—were used as controls.

Specifically, total SMAD2 and the phosphorylation of SMAD2 in T cells exposed or not to TGFβ-1 (10 ng/mL) was measured by flow cytometry. Phospho-flow was performed on three batches of process-representative TCR-T cells test articles to demonstrate that TSC-201-B0702 TCR-T cells, which express DN-TGFβRII, are resistant to the immunosuppressive effect of physiological levels of TGFβ. Process-similar untransfected T

cells were included as positive control for phosphor-SMAD2 induction, as these test samples lack DN-TGFβRII and have intact TGFβRII-mediated pathways. Resistance to TGFβ-1 signaling was assessed by flow cytometric histogram analysis of Phospho-SMAD2 in TCR-T cells ($CD34^+$) compared to untransfected controls ($CD34^-$).

Process representative test Samples TSC-201-B0702 from batches PD353, PD354, and PD355 were tested alongside donor-matched process similar bench-scale UTF controls. The process representative UTF controls had poor viability <20% and were therefore not used in this study. T cells from batch PD355 did not have a donor-matched bench-scale process similar UTF control available. Instead, two non-donor matched bench-scale process similar UTF controls were included, to control for the effect of TGFβ-1 signaling in test samples with endogenous TGFβRII receptor (RF2959-UTF and D6466-UTF; similar results were obtained with both UTFs). TCR-T cells and UTF control test samples were thawed as described above. After overnight recovery, test samples were harvested and washed by centrifugation with serum-free RPMI-1640 then resuspend in serum-free RPMI-1640 to the desired cell density of 5E6 viable cells/mL. Test samples were transferred to the 96-well v-bottom assay plate, 5E5 total viable cells per well and placed in the 37°C incubator for 1 hour to reduce basal levels of phosphorylation.

Serum-starved test samples were untreated or treated with TGFβ-1 (10ng/mL). The cells were simultaneously stained with CD34(QBEND10)-AF488 to distinguish engineered cells ($CD34^+$) from non-engineered in the TCR-T cells, and with LIVE/DEAD fixable yellow dead cell stain to distinguish live from dead cells during analysis. Master mixes and fluorescence minus one (FMO) controls were prepared with the critical materials (see Table 5); single-color control master mixes were also prepared. The assay plates were placed in the 37°C incubator for 1 hour.

Test samples were stained accordingly: (1) Untreated/CD34/LIVE/DEAD; (2) TGFβ-1-treatement/CD34/LIVE/DEAD; (3) Untreated/CD34 only; (4) TGFβ-1-treatement/CD34only; (5) Untreated/LIVE/DEAD only; and (6) TGFβ-1-treatement/LIVE/DEAD only. Some CD34 signal in the extracellular epitope was lost following fixation/permeabilization. As a control for $CD34^-$signal loss in permeabilized cells, control wells containing live cells stained only for CD34 were included in the assay plate. These wells proceeded the same temperature incubations and washes as test samples; however, PBS was added during the fixative and permeabilization steps. Fixative was added to test samples in the assay plate to maintain their phosphorylation state. After a 15-minute 37°C incubation, the assay plates were washed by centrifugation and the cells were then

permeabilized for 20 minutes on ice to enable intracellular immunostaining of Phospho-SMAD2 (Ser465/Ser467) or Total SMAD2 Rabbit mAb. The assay plates were incubated in the dark at room temperature for 60 minutes. The assay plates were washed by centrifugation twice. The cell supernatants were resuspended in antibody dilution buffer and prepared for acquisition. Experimental Test samples: non-treated TCR-T cells and TGFβ-1(10ng/mL) treated TCR-T cells; Experimental Controls: non-treated UTF controls (endogenous TGFβRII) and TGFβ-1(10ng/mL) treated UTF; Gating Controls: FMO-Phospho/Total SMAD2 (untreated or treated) and LIVE/DEAD only; Staining Control: Staining of Total SAMD2 protein (untreated and treated, staining with CD34 and LIVE/DEAD), Live (unfixed/unpermed) TCR-T cells stained with CD34 only (untreated), Staining of CD34 only (untreated and treated), Staining of Total SMAD2 protein only (untreated and treated) and unstained (untreated and treated).

Data acquisition was performed on a CytoFLEX S. Flow cytometric analysis was performed with FlowJo v10.8.1, the statistics were exported to Microsoft 365 Excel 2023 and analyzed. Phospho-SMAD2 levels were assessed by comparing the TGFβ-1 treated mean fluorescence intensity (MFI) to the untreated MFI. Gates were arranged in accordance with the FMO-Phospho-SMAD2 control. A signal to noise (S/N) greater than 2 indicates true Phospho-SMAD2 signal; levels above background (untreated).

Engineered TCR-T cells were gated based on their CD34(QBEND10) tag, and gates were arranged according to the FMO-CD34 control (LIVE/DEAD only). Untransfected control cells (UTF) did not have any CD34 positive cells therefore were gated on CD34 negative populations.

Based on flow cytometric histogram analysis, percentage of Phospho-SMAD2 was reported in TGFβ-1 stimulated and non-stimulated for both TCR-T cells (CD34 positive cells) and UTF controls (CD34 negative cells). Test samples were analyzed further by measuring the total SMAD2 levels. The measure of total SMAD2 protein levels controls for the levels of SMAD2 present in the samples, supporting the accuracy of any increase or decrease in the phosphorylation of SMAD2.

As illustrated in Figure 8, histogram analysis of Total SMAD2 and phospho-SMAD2 was performed on untreated (filled histograms) and TGFβ-1-treated (open histograms) TSC-201-B0702 TCR-T cells (purple, gated on $CD34^+$) and their donor-matched UTF controls (black, gated on $CD34^-$). In the bottom row, the mean fluorescence intensity (MFI) of Total SMAD2 was similar for all four test sample conditions. The ratio of total SMAD2 in TGFβ-1 treated versus untreated T cells remained below 2. This indicates total SMAD2 protein levels

were comparable across every condition. Accordingly, when measuring the phosphorylation of SMAD2, any differences in signal was attributed to a difference of the phosphorylation state of the protein rather than to a difference in the amount total SMAD2 protein in the cells.

When considering phospho-SMAD2, no signal was detected in untreated test articles (filled histograms). Upon TGFβ stimulation, phospho-SMAD2 was detected in 97.27%, 97.73% and 97.70% of the UTF control T cells (grey, open histograms; Figure 8).

These data confirmed that TGFβ was effectively inducing downstream signals in the T cells, as virtually all the SMAD2 protein was phosphorylated in the UTF control T cells lacking DN-TGFβRII. Comparatively, TGFβ mediated stimulation of SMAD2 phosphorylation in the $CD34^+$ fraction of 3 batches of process-representative TSC-201-B0702 TCR-T cells (expressing DN-TGFβRII) was reduced, with only 44.90%,43.03%, and 42.17% of phopho-SMAD2-positive cells detected for batches PD353, PD354, and PD355 (green, open histograms), respectively (representing a decrease of positive signal by 53.84%, 55.97% and 56.84%, respectively, when compared to the UTF controls). In addition to the sharp decrease in the proportion of cells displaying phosphorylated SMAD2 protein, the intensity of the signal in the phospho-SMAD2 positive fraction was also consistently reduced when comparing DN-TGFβRII-expressing TCR-T cells and UTF control T cells. It is worth noting that most of the TCR-T cells expressing DN-TGFβRII essentially failed to phosphorylate SMAD2 in response to TGFβ as revealed by a peak of cells with phospho-SMAD2 signal aligned with the signal measured in unstimulated cells.

To further validate that the phospho-SMAD2 assay correlates with a functional output, the ability of process-representative TSC-201-B0702 TCR-T to maintain their target-dependent stimulation of IFN-γ secretion in the presence of TGFβ was evaluated. Besides four batches of process-representative TSC-201-B0702 TCR-T cells (PD354, PD354, PD355 and PD354), two batches of bench-scale engineered TSC-201-B0702 TCR-T cells lacking DN-TGFβRII were included in this cell-based functional assay as controls with intact TGFβ signaling. Process-representative TSC-201-B0702 TCR-T cells (expressing DN-TGFβRII) were found to be more resistant to TGFβ-mediated inhibition of target-induced IFN-γ secretion compared to the bench-scale TSC-201-B0702 TCR-T cells lacking DN-TGFβRII. These data further support that the reduced phospho-SMAD2 induction observed in DN-TGFβRII expressing TCR-T cells upon stimulation with TGFβ correlates with resistance to TGFβ mediated inhibition of T cell function.

Overall, these observations support the conclusion that process-representative TSC-201-B0702 TCR-T cells present a reduced sensitivity to TGFβ signaling when compared to cells lacking DN-TGFβRII.

Example 4: *In vivo* efficacy of TSC-201-B0702

The *in vivo* anti-tumor activity and tolerability of a representative number of independent donor-derived batches of TSC-201-B0702 TCR T cells prepared as described in Example 3 above and administered intravenously in a representative cancer model (subcutanouse xenograft model of A101D β2M tumor in female NCG mice) were evaluated. TSC-201-B0702 TCR-T cells displayed a strong and potent anti-tumor activity with all the three batches of TSC-201-B0702 TCR-T cells tested compared to both the vehicle and respective UTF control groups, based on area under the curve (AUC) data corresponding to tumor growth curves for individual mice in the study (see Figure 9), and the tumor growth inhibition (TGI) data. Based on body weight loss and clinical obsercations, all the treatment groups were found to be well tolerated. No overt signs of toxicity were observed and there were no apparent treatment-related deaths.

In particular, efficacy of three batches of TSC-201-B0702 from different donors was compared to non-engineered (untransfected [UTF]) control T cells from matching donors, and vehicle (PBS) treatments against the A101D β2M tumor cell line implanted in NCG female mice. The A101D β2M cell line is derived from A101D parental cell line, a skin melanoma. The A101D β2M cell line was generated by overexpressing β2M in A101D cells to stabilize class I HLA on these tumor cells. The A101D β2M tumor cells endogenously express MAGE-C2 and HLA-B*07:02 and were also confirmed to be recognized by TSC-201-B0702 TCR-T cells, *in vitro* (see Example 3 above). Tumor cells were inoculated subcutaneously in the right flank of the animals. Once tumor engraftment was successful (tumors reaching 70 mm^3 on average), animals were randomized into different treatment groups, and received two doses of TSC-201-B0702, of corresponding UTF control T cells, or of vehicle (PBS), 7 days apart. Different readouts were gathered over time: (1) anti-tumor efficacy was evaluated by biweekly tumor volume measurements; (2) biweekly body weight measurements were recorded alongside any clinical observations to gauge any toxicity related to TSC-201-B0702 injection.

Materials and methods used for *in vivo* efficacy tests are described herein. Briefly, the A101D β2M cell line derived from A101D parental cells is a skin melanoma purchased from ATCC (catalog # CRL-7898) and cultured according to the manufacturer's

recommendations. The cell culture medium used to grow this cell line was DMEM supplemented with 10% FBS (Gibco). The transgene for β2M was introduced into A101D tumor cells via lentiviral transduction, followed by enrichment of A101D β2M cells by using Blasticidin (40 μg/mL). The tumor cells were maintained at log phase growth in tissue culture flasks in a humidified incubator at 37°C, in an atmosphere of 5% CO2 and 95% air.

On the days of dosing, cryo-bags (75 mL) of cryopreserved TSC-201-B0702 TCR-T cells and UTF control T cells from matching donors were thawed (one group at a time) using 37°C water bath and the T cells were transferred to thawing media consisting of X-VIVO™ 15 media (Lonza Cat# 04-418Q), and 5% heat-inactivated human serum (Sigma Cat No. H3667). The T cells were then washed and resuspended in appropriate volume of sterile PBS to obtain dosing suspension at concentrations of 2E7 viable $CD34^+$ cells per 0.1 mL. The total amount of live T cell injected was adjusted to account for $CD34^+$ purity. The number of UTF control T cells injected was adjusted to match the total number of live cells needed for TSC-201-B0702 of the matching batch.

One hundred (100) female NOD-*Prkdc*^{em26Cd52}*Il2r*^{gem26Cd22}/NjuCrl (NCG) mice were ordered and inoculated with A101D β2M tumor cells for potential assignment to the study. The mice were 7 weeks old, with body weights ranging from 17.7 to 28.27g at the beginning of the study. Treatment groups were created to test 3 batches of TSC-201-B0702 TCR-T cells according to the study design (Table 6). Excess animals bearing A101D β2M xenografts were euthanized.

A101D β2M tumor cells used for subcutaneous xenografts were harvested during log phase growth and washed in PBS. Mice were inoculated subcutaneously with 100 μL of a mix of 50% matrigel /50% medium containing 2E6 live A101D β2M cells to establish the tumors. Tumors were measured by caliper bi-weekly, starting 5 days post inoculation and continuing until the end of the study (47 days post inoculation, *i.e.*, study Day 42). Randomization was conducted once the mean tumor volume for all the groups reached ~70 mm^3 (with individual values ranging from 49.95 mm^3 to 93.73 mm^3). Randomization corresponds to Day 0 of study.

Animals received repeat doses of TSC-201-B0702 on Day 1, and Day 8 according to the study design presented in Table 6, and Figure 9. The dosing volume was 100 μL, administered intravenously (*i.v.*). Group 1 received injections of vehicle control (PBS); Group 3 received injections of TSC-201-B0702 from batch PD353 (2.0E7 live $CD34^+$ cells corresponding to 2.64 E7 total live cells); Group 5 received injections of TSC-201-B0702 from batch PD354 (2.0E7 live $CD34^+$ cells corresponding to 2.72E7 total live cells); and

Group 7 received injections of TSC-201-B0702 from batch PD355 (2.0E7 live $CD34^+$ cells corresponding to 2.73E7 total live cells). The number of UTF control T cells injected for each group was matched with the total live T cells injected for the respective TSC-201-B0702 treatment groups: Group 2, Group 4, and Group 6 received live untransfected (UTF) control T cells from batch PD353 (2.64E7 live T cells), PD354 (2.72E7 live T cells) and PD355 (2.73E7 live T cells), respectively.

Table 6: Experimental design and reagents

Experimental design

Gr.	N	Agent	Formulation dose (Live $CD34^+$)	Route	Schedule
1	12	Vehicle (PBS)	NA	*i.v.*	Days 1,8
2	12	PD353-D UTF	2E7 cells/animal	*i.v.*	Days 1,8
3	12	PD353-F TSC-201-B0702	2E7 cells/animal	*i.v.*	Days 1,8
4	12	PD354-D UTF	2E7 cells/animal	*i.v.*	Days 1,8
5	12	PD354-F TSC-201-B0702	2E7 cells/animal	*i.v.*	Days 1,8
6	12	PD355-D UTF	2E7 cells/animal	*i.v.*	Days 1,8
7	12	PD355-F TSC-201-B0702	2E7 cells/animal	*i.v.*	Days 1,8

Reagents

Media	Vendor	Catalog Number
DMEM	ATCC	30-2002
Fetal Bovine Serum (Heat-Inactivated)	Gibco	A38400
DPBS (Dulbecco's phosphate-buffered saline)	Gibco	14190-136

Reagent	Vendor	Catalog Number
0.4% Quatricide	Medline	N/A (From Explora)
Wescodyne	West Penetone	1511
DPBS	Gibco	14190-136
FBS	Gibco	35050-001
DMSO	Sigma	D265-100ml
GlutaMax (100x)	Gibco	35050-061
DMEM	ATCC	#30-2002
ViaStain AOPI Staining Solution	Nexcelom Bioscience	CS2-0106-25mL
70% Isopropanol	Decon Laboratories Inc	8601
BD Cytofix	BD	554655

Throughout the study, mice were observed for general health/mortality and moribundity twice daily, once in the morning and once in the afternoon. Cage-side observations occurred daily. Mice were not removed from their cage during observation, unless necessary for identification or confirmation of possible findings. The mice were also observed for overt signs of any adverse treatment-related (TR) side effects, body weight and tumor measurement activities. Body weights were recorded on days 0, 3, 7, 10, 14, 17, 22, 24, 28, 31, 35, 38, and 42 days of the study. Tumors were measured by caliper (mm^3 units) biweekly, until Day 42 of the study. The tumor volume and body weight of individual mice were recorded twice a week in the software Overwatch. The greatest longitudinal diameter (length [L]) and the greatest transverse diameter (width [W]) were determined and reported into the Overwatch software. The tumor volume was estimated using the ellipsoidal formula $V = (W^2 \times L)/2$.

Area under the curve (AUC) was used to represent the entire tumor growth curve for a mouse by a single integrated numerical value, using Graphpad prism (Duan *et al.*, (2012) *Journal of Immunological Methods* 382:224-228). This allows easier comparison between different experimental groups and provides a better idea about the antitumor activity of any treatment, in studies involving large numbers of mice. The AUC was determined for individual mice in this study, considering their respective tumor growth curves from Day 0 to Day 28, as 100% of animals were alive in all the groups until this timepoint.

Tumor growth inhibition (TGI) was calculated by comparing the mean tumor volume of animals in different groups at the latest time point of the study when all the mice are alive. In the present study all the mice were alive until Day 28 of the study. Hence, TGI was

calculated taking into consideration the mean tumor volume of mice of different groups on Day 28 of the study. The TGI was calculated by using the following formula.

TGI = 100% x (mean tumor volume of control – mean tumor volume of treatment) / mean tumor volume of control.

The endpoint of the experiment was death or moribundity due to tumor progression, ulceration in the tumor, tumor volume reaching 2000 mm^3, or the last day of the study (Day 42 of the study, corresponding to day 47 post-inoculation of tumor cells).

Animals were monitored individually for signs of moribundity, decreases in body weight and tumor progression and classified as death on study. The time to endpoint (TTE), in days, was recorded for each mouse that died of its disease or was euthanized due to tumor progression. Any animal classified as having died from treatment-related (TR) causes was assigned a TTE value equal to the day of death. Any animal that did not appear moribund but was euthanized due disease progression as supported by necropsy observations, was recorded as a non-treatment-related death due to tumor invasion or metastasis (NTRm) and was included in the data analysis. Any death due to unknown causes (NTRu) or due to an accident or error (NTRa) was excluded from TTE calculations and all further analyses.

Pairwise comparisons of area under the curve (AUC) and tumor growth inhibition (TGI) were performed, where the mean AUC and TGI for the animals of UTF control groups was compared with that of corresponding TSC-201-B0702 TCR-T cell treatment group, respectively. The AUC and growth rate estimates were assumed to be normally distributed, and unpaired t-test was used to check if there was a statistically significant difference between the control group and TSC-201-B0702 TCR-T cell treated group animals. Statistical analyses were performed using Student's t-test in GraphPad Prism® 10.0 software and P-values < 0.05 were considered statistically significant.

a. TSC-201-B0702 TCR-T cells exhibit *in vivo* anti-tumor efficacy

The efficacy of TSC-201-B0702 TCR-T cells was evaluated against the A101D β2M tumor model in NCG female mice. Three batches of process-representative TSC-201-B0702 material were compared to untransfected (UTF) control T cells from matching donors, and to vehicle control (PBS) treatment. The mice were randomized to produce 7 groups of 12 mice each (see Table 6), when the mean tumor volume for all the experimental groups was ~70 mm^3 with individual values ranging from 49.95 mm^3 to 93.73 mm^3. The mice of individual experimental groups were dosed with one of the 3 batches of process representative TSC-201-B0702 TCR-T cells, or respective UTF control T cells or Vehicle (PBS) twice, at one

week interval (Day 1, and Day 8 of the study). The TSC-201-B0702 treatment groups were injected with 2E7 live $CD34^+$ (*i.e.*, engineered) TCR-T cells on each of the two dosing time points. Cellular dose was adjusted for the purity of the material and corresponded to 2.64E7 total T cells for batch PD353, 2.72E7 total T cells for batch PD354, and 2.73E7 total T cells for batch PD355, respectively. The number of UTF control T cells injected for each UTF control group matched the total T cells injected for the respective TSC-201-B0702 treatment groups. The purity of engineered TCR-T cells (~75% of $CD34^+$) and viability (75% - 80%) of the three batches of TSC-201-B0702 TCR-T cells were overall comparable.

As evident from the tumor growth curve profiles for different experimental groups in Figures 10A-10E, animals for control groups (Group 1 [Vehicle], 2, 4, and 6 [UTF control T cells groups from batches PD353, PD354, and PD355, respectively]) presented larger tumors with mean tumor volumes of 1608.71 mm^3,1760.85 mm^3, 1914.14 mm^3, and 1499.84 mm^3, respectively, at the end point of the study. The Groups 3, 5, and 7 that received *i.v.* injections of TSC-201-B0702 TCR-T cells from batch PD353, PD354, and PD355, respectively showed appreciable antitumor activity with reduced mean tumor volumes of 896.03 mm^3, 742.18 mm^3, and 775.85 mm^3, respectively, at the endpoint of the study.

The %TGI was calculated by considering the tumor growth in control groups (Vehicle, and corresponding UTF control groups) and the three batches of TSC-201-B0702 TCR-T cell treatment groups on Day 28 of the study, when 100% of the mice were alive in the groups. When compared to the corresponding UTF control groups, the %TGI was found to be 40.73%, 56.49%, and 54.37% for TSC-201-B0702 TCR-T cell treatment groups from PD353, PD354, and PD355 batches, respectively. Similarly, when compared to the vehicle control group, the %TGI was found to be 32.71%, 57.41%, and 54.32% for TSC-201-B0702 TCR-T cell treatment groups from PD353, PD354, and PD355 batches, respectively. This data suggests that TSC-201-B0702 TCR-T cells present antitumor activity *in vivo*.

Tumors in UTF-treated control groups (Groups 2, 4 and 6) grew at a similar rate as the vehicle-treated group (Group 1), with -13.51% tumor growth inhibition for Group 2, 2.11% and -0.11% tumor growth inhibition for Groups 4, and 6, respectively.

Contrary to the %TGI which is calculated from the tumor volumes at one time point (Day 28 of the study, when all the mice were alive), the AUC is calculated from the tumor progression curve, representing the change in tumor volume of individual mice over entire course of the study (from Day 0 to Day 28, when all the mice were alive). Therefore, the AUC values represent the entire tumor growth curve for each mouse by a single integrated numerical value and is expected to be more comprehensive to assess the anti-tumor activity

of the TCR-T cells. The AUC values of the TSC-201-B0702 TCR-T cell treated groups from all the 3 batches (PD353, PD354, and PD355) were found to be significantly lower than that of corresponding UTF control groups (P-values <0.0001 for all the 3 groups), clearly demonstrating the antitumor activity of TSC-201-B0702 TCR-T cells against A101D β2M tumor, *in vivo* (Figure 10B).

Further, whereas several animals consistently reached tumor volume endpoint in the control groups, every animal in the TSC-201-B0702 treated groups survived to the end of the study (excluding animals which were taken out of study due to ulceration). These observations reinforce the conclusion that TSC-201-B0702 TCR-T cells present a potent antitumor activity against A101D β2M tumors, *in vivo*.

b. TSC-201-B0702 TCR-T cells exhibit *in vivo* delay of mortality

There were no apparent treatment-related deaths. The body weight appeared consistent across groups throughout the course of the study, regardless or treatment regimen (Figure 11), indicating no overt signs of toxicity in the treated groups.

Example 5: Identification of putative off-targets for TSC-201-B0702

Any potential off-targets of the therapeutic TCR used in TSC-201-B0702 using TScan's proprietary genome-wide screen called *TargetScan*. Off-target epitopes recognized by a TCR have traditionally been predicted using computational algorithms or screening positional scanning libraries. TScan takes an unbiased and comprehensive approach by using a proprietary genome-wide safety screening platform: the Target Scan screen (Kula *et al.* (2019) *Cell* 178:1016-1028; Ferretti *et al.* (2020) *Immunity* 53:1095-1107). This approach enables identification of epitopes recognized by a given TCR even if they have low sequence homology with the on-target epitope and are therefore unlikely to be predicted using computational methods or a more focused mutational screen (Figure 12).

The *TargetScan* screen is a high-throughput cell-based technology that enables comprehensive identification of the natural targets and potential off-targets of $CD8^+$ T cells in an unbiased, genome-wide fashion. Briefly, $CD8^+$ T cells expressing the TCR of interest (in this case, the recombinant TCR for TSC-201-B0702; hereafter referred to as mechanistically representative TSC-201-B0702 TCR-T cells) are co-cultured with HEK293T target cells that express a genome-wide library of protein fragments and have been engineered to express the HLA of interest (in this case HLA-A*07:02). Target cells also express a reporter that becomes fluorescent in the presence of granzyme B activity. Each target cell in the library

also expresses a different ~90-amino acid protein fragment (also known as a 90mer). Collectively, the library expresses fragments that span every human protein in the proteome and include all common single nucleotide polymorphisms. These fragments are processed naturally by the target cells, and peptides derived from the fragments are displayed on class I MHCs (in this case HLA-B*07:02) on the cell surface. If an engineered CD8+ T cell encounters a peptide/MHC target that it recognizes in the co-culture, the T cell secretes cytotoxic granules into the target cell, triggering the target cell to become fluorescent. Early apoptotic cells are isolated from the co-culture and fluorescent cells are further purified by fluorescence-activated cell sorting. The expression cassettes of the sorted cells are then sequenced, revealing the identities of the protein fragments expressed in those cells. The protein fragment encoding an epitope recognized by the TCR would exhibit a fold increase in the sorted cells compared to library input.

The Target Scan screen is enabled by two proprietary components: (i) TScan's granzyme B-activated infrared fluorescent protein (IFP) reporter allows cells targeted by a specific TCR to be identified, sorted, and analyzed; and (ii) TScan's target-cell libraries encompass a broad range of protein fragments that provide comprehensive, proteome-wide evaluation of all potential targets and off-targets of a given TCR. The primary screen used to analyze the therapeutic TCR of TSC-201-B0702 was performed using a proteome-wide library comprising ~500,000 clones. As part of the *TargetScan* screening process, TScan routinely analyzes the starting library by next generation sequencing. This effort verified that >89% of the clones in the library are represented in the screens. On average, each clone is typically present at >10,000 copies in the co-culture.

Besides confirming that the mechanistically representative TSC-201-B0702 TCR-T cells recognize its target MAGE-C2 epitope, the data presented in this report demonstrate that the proteome-wide screen identified CLCH1 and SLC26A7 as putative off-targets for the TSC-201-B0702 TCR. Follow up experiments using functional assays were performed to assess the reactivity of process representative TSC-201-B0702 TCR-T cells against these putative off-targets to determine the risk (or lack thereof) of off-tumor reactivity. These follow up functional data are presented in Example 7 below. These follow-up efforts confirmed that TSC-201-B0702 TCR-T cells lack reactivity to cells endogenously expressing the putative off-target proteins identified here, indicating that none of these putative off-targets pose a risk of off-tumor reactivity for TSC-201-B0702. Instead, the TCR-T cells present a selective reactivity to the MAGE-C2-derived epitope presented on HLA-B*07:02.

First, the lentivirus was packaged and the viral titer was determined. Lenti-X cells (Takara Bio USA, Mountain View, CA) were plated at 75% confluency and transfected using jetPRIME transfection reagent (Polyplus, Illkirch, France). Briefly, lentiviral constructs were mixed with packaging plasmids (pREV/pTAT/pVSVG/pGAGPOL) and incubated with jetPRIME reagent according to the manufacturer's protocol, and Opti-Pro SFM medium was added at 24 hours post-transfection. Viral supernatants were harvested 48 hours after transfection. Viral supernatants to produce mechanistically representative TSC-201-B0702 TCR-T cells were concentrated using either Vivaspin 20 centrifugal concentrators or Vivaflow 50 cassettes (Sartorius, Bohemia, NY). Virus titer for supernatants was determined by RT-qPCR using Luna® Universal One-Step RT-qPCR Kit (New England BioLabs) on a QuantStudio 7 Pro (ThermoFisher Scientific). Briefly, virus was diluted in PBS, lysed and 2µl of the lysed virus was used in RT-qPCR according to manufacturer's instructions. Standard curve was generated using serial dilutions of virus with known titer. Titer was expressed as transduction units (TU)/ml. For peptide library virus, lentiviral titer was determined by puromycin colonies formation using Lenti-X™ cells transduced with serial dilution of viral supernatant. To quantify viral titers, puromycin resistance colonies were selected 48 hours post-transduction. Puromycin resistance colonies were visualized by crystal violet staining and counted. Titer was calculated as the colony forming unit as TU/ml using the formula: TU/ml = Number of puromycin resistance colonies x dilution factor x 1000.

Reporter cells were prepared. Briefly, reporter cells were generated by knocking endogenous HLA-A/B/C out in HEK293T cells using CRISPR and engineered to express a granzyme-activated infrared fluorescent protein (IFP) (Kula *et al.* (2019) *Cell* 178:1016-1028) and a granzyme-activated scramblase (Ferretti *et al.* (2020) *Immunity* 53:1095-1107). Reporter cells for screening mechanistically representative TSC-201-B0702 TCR-T cells were engineered to express HLA-B*07:02.

The human genome-wide peptide library was generated by tiling across the human genome coding sequences spanning all proteins of the human genome with overlapping 90-mer amino acid tiles. The tiles were synthesized on a silicon chip (Twist Bioscience) and cloned into a lentivirus expression vector.

Reporter cells were transduced with lentiviral particles to express the PEPTIDOME vSAFE-SNP library at an MOI of 5 in complete DMEM (1X DMEM supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin). This library is comprised of >460,000 constructs (tiles) comprised of 90 amino acids each, representative of

the human proteome. The library also contains ~40,000 tiles representative of single-nucleotide polymorphisms (SNPs) present in >1% frequency of the US population. The day after transduction, complete DMEM media was refreshed, and cells were frozen in complete DMEM supplemented with 10% DMSO until needed.

Mechanistically representative TSC-201-B0702 TCR-T cells were generated. Briefly, Primary $CD8^+$ T cells from a single donor were isolated using the StraightFrom Leukopak CD8 Microbead Kit (Miltenyi Biotec) according to the manufacturer's protocol. Isolated cells were frozen in CryoStor® CS10 (Stem Cell Technologies) and stored in liquid nitrogen until use. On Day -1, $CD8^+$ T cells were thawed, washed with complete T cell medium (RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum [FBS], 100 IU/mL penicillin, 100 µg/mL streptomycin, recombinant human IL-2 [50 U/mL, PeproTech, Cranbury, NJ], recombinant human IL-15 [5 ng/mL, R&D Systems], and recombinant human IL-7 [5 ng/mL, R&D Systems]), seeded at 1.0E6/mL, and rested overnight at 37°C. On Day 0, $CD8^+$ T cells were washed and resuspended in fresh T cell medium and activated using ImmunoCult™ human CD3/CD28/CD2 T cell activator (5 mL/1.0E6 $CD8^+$ T cells, Stem Cell Technologies). On Day 1, cells were washed and resuspended in fresh complete T cell medium and transduced with lentiviral particles to express the recombinant TCR for TSC-201-B0702.

On Day 2, cells were washed and resuspended in fresh complete T cell medium and expanded until Day 5 in G-Rex® 6-well plates (Wilson Wolf). On Day 5, cells were isolated using manual MACS. Cells were harvested and resuspended in MACS running buffer (Miltenyi Biotec) and incubated with CD34 beads, FC block and MACS buffer at 4°C for 30 minutes. Cells were washed with MACS buffer, spun down and resuspended in MACS buffer and loaded onto magnetic LS columns for isolation. The isolated cells were resuspended in fresh complete T cell medium and expanded in G-Rex® 10 flasks (Wilson Wolf) until Day 12, at which point the cells were frozen down in CryoStor® CS10 and stored in liquid nitrogen until used.

Mechanistically representative TSC-201-B0702 TCR-T cells were thawed and restimulated (further expanded) by coculturing T cells with irradiated (60 grays) allogeneic PBMCs in the presence of 0.1 µg/mL anti-CD3 (OKT3, eBioscience) and 50 U/mL recombinant IL-2 (Peprotech) in fresh T cell medium (RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum [FBS], 100 IU/mL penicillin, 100 µg/mL streptomycin, recombinant human IL-2 [50 U/mL, PeproTech]) in G-Rex® 100 flasks (Wilson Wolf). 50 U/mL recombinant IL-2 was added to expanding cells every other day until Day 6. On Day

6, half of culture medium was replaced with fresh T cell medium containing 50 U/mL recombinant IL-2. Cells were used for screens on Day 7.

TargetScan screen was then carried out. Specifically, on Day 5 of restimulation of mechanistically representative TSC-201-B0702 TCR-T cells, reporter cells (expressing the peptidome library) were labeled with Cell Trace Violet (Thermo Fisher) for 10 minutes at room temperature. Labeling reaction was quenched with a 5X excess of complete DMEM media (1X DMEM supplemented with 10% FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin). After centrifugation, 4E8 labelled reporter cells were seeded in a CellSTACK flask.

On Day 6 of restimulation, T cells' reactivity against the MAGE-C2 epitope and the ability of the reporter cells to respond to granzyme B signaling were confirmed prior to engaging in the screen co-culture. A fraction of reporter cells was pulsed with 1μM of MAGE-C2 peptide (RAREFMELL, Genscript) for one hour. T cells were added to reporter cells at four effector to target (E:T) ratios (2:1, 1:1, 1:2, 1:4) in triplicate. After four hours of incubation at 37°C, cells were resuspended by pipetting up and down before data acquisition on a CytoFLEX flow cytometer (Beckman Coulter).

On Day 7 of restimulation, mechanistically representative TSC-201-B0702 TCR-T cells were added to library-transduced reporter cells and incubated at 37°C for four hours. After incubation, all cells were harvested by trypsinization and centrifugation, resuspended in 1X Annexin V binding buffer (Miltenyi Biotec), and centrifuged. Cells were resuspended with Annexin V magnetic microbeads (Miltenyi Biotec) in 1X Annexin V binding buffer (1 mL microbeads in 9 mL Annexin V binding buffer per 1E9 total cells) and incubated at room temperature for 15 minutes. Cells were washed with 5X volume of Annexin V binding buffer and centrifuged. Cells were resuspended in Annexin V binding buffer and then divided to 2 replicates and filtered using a 70μM cell strainer (Corning). Annexin V-labeled cells were positively selected using an AutoMACS Pro (Miltenyi Biotec). The elution of each replicate was further divided over four sort replicates for a total of 8 technical replicates per screen. IFP^+ cells were sorted using a MoFlo Astrios EQ cell sorter (Beckman Coulter). The E:T ratio for the mechanistically representative TSC-201-B0702 TCR-T cells in this off-target screen was 2:1.

Genomic DNA (gDNA) was extracted from sorted cells using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific). The antigen cassette was amplified from the extracted gDNA by PCR, then appended with sequencing adaptors and sample-specific

index sequences in a second PCR reaction. Amplicons were sequenced on an Illumina® NextSeq using the standard Illumina sequencing primer.

Nucleotide sequences were mapped to individual nucleotide tiles. The proportion of read counts for each tile was calculated for each screen replicate (n=8) and for the input for each pool of transduced reporter cells. Enrichments of each tile were calculated by dividing the proportion of the tile in the screen replicate by the proportion of the tile in the input library. A modified geometric mean of the enrichment of an identical tile across the 8 screen replicates was used to identify reproducible screen hits. Any sequence which showed an enrichment over background across multiple 90-mer tiles was considered a 'hit'.

Table 7

Cell Lines

Cell	Vendor	Catalog Number
HEK293T	ATCC	CRL-3216
Lenti-X	Takara Bio	632180
Jurkat	ATCC	TIB-152
FTC133	Sigma Aldrich	94060901
A101D	ATCC	CRL-7898
A2058	ATCC	CRL-11147
COLO783	DSMZ	ACC 257

Media and Supplements

Media and Supplements	Vendor	Catalog Number
Penicillin streptomycin	Gibco	15149-122
RPMI-1640 Medium	ATCC	30-2001
Fetal Bovine Serum (FBS), heat inactivated	Gibco	A38401012
DMEM (1x) [+] 4.5 g/L D-glucose, [+] L- glutamine, [-] Sodium Pyruvate:	Gibco	11965
1x DPBS (no Ca2+/Mg2+)	Gibco	A3840102
MACS Running buffer	Miltenyi	130-091-221
EasySep Buffer	StemCell Technologies	20144
CryoStor CS10	StemCell Technologies	07930
Lymphocyte Separation Medium	Corning	25-072-CV

Cytokines

Cytokines	Vendor	Catalog Number
Recombinant human IL-2	Peprotech	200-02
Recombinant human IL-7	R&D Systems	207-II-005
Recombinant IL-15	R&D Systems	247-ILB-005

Kits

Kits	Vendor	Catalog Number
Straight From Leukopak CD8 Microbead Kit	Miltenyi Biotech	130-117-019
CD34 Microbead Kit	R&D Systems	130-046-703
Annexin V Microbead Kit	R&D Systems	207-II-005

Antibodies and Staining Reagents

Antibodies and Staining Reagents	Vendor	Catalog Number
Anti-human HLA-A, B, C PE (clone: W6/32)	BioLegend	311406
Cell Trace Violet (CTV)	Fisher Scientific	C34571
Anti-human CD32 (Fc gamma RII) Blocker	Stemcell Technologies	18520
Anti-human CD3 (clone: OKT3)	Invitrogen	16-0037-85
Anti-human CD34-Alexa Fluor 488 (clone:Qbend10)	R&D Systems	310916
ImmunoCult Human CD3/CD28/CD2 T cell activator	StemCell Technologies	10970
DAPI (4',6-diamidino-2-phenylindole)	Thermo Scientific	62248
ViaStain AO/PI staining solution in PBS	Nexcelom Bioscience	CS2-0106-5ml

Dextramers and Peptides

Dextramers and Peptides	Vendor	Catalog Number
MAGE-C2 peptide (RAREFMELL)	GenScript	custom
HLA-B*07:02/ RAREFMELL Dextramer - PE	Immudex	custom

Genomics Reagents

Material	Vendor	Catalog Number
GeneJet Genomic DNA Purification kit	Thermo Fisher Scientific	K0722

The results of the *TargetScan* screen are shown in Figure 13 in the form of a dot plot where each of the ~500,000 tiles forming the proteome-wide library are ordered along the X-axis, and the fold-enrichment for each tile, compared to the input library, is indicated on the Y axis. Out of the ~500,000 clones expressing protein fragments that collectively spanned every human protein and covered all common SNPs, only 8 clones were enriched when the mechanistically representative TSC-201-B0702 TCR-T cells were evaluated. Besides the 2 clones expressing tiles corresponding to the portion of MAGE-C2 producing the HLA-B*07:02-restricted epitope, the remainder of the enriched clones contained overlapping protein tiles derived from 2 different genes representing potential off-targets for TSC-201-B0702: CLHC1 and SLC26A7. Note that the sequences of CLHC1 and SLC26A7 identified as containing the putative off-target epitope for TSC-201-B0702 share some sequence similarity with the known peptide derived from MAGE-C2 recognized by TSC-201-B0702

(bolded and underlined in Table 8). Also detected in the screen are 2 SNP clones each expressing a unique concatemer of 4 gene segments that contain a SNP. These clones do not represent potential off targets for TSC-201-B0702 because each true SNP is represented 3 times in the peptide library, but only one clone scores in this *TargetScan* screen. Further, 4 separate gene segments are included on one peptide tile generating a unique artificial sequence at the junction of each gene segment (junctions shown as "|" in Table 8). For the enriched SNP tiles, the known peptide derived from MAGE-C2 recognized by TSC-201-B0702 aligns with a region spanning the junction of two gene segments included on the peptide tile (shown bolded in Table 8). The AA sequences of the enriched clones are shown in Table 8 with the on-target MAGE-C2 epitope (RAREFMELL) bolded and italicized across 2 scoring clones.

Table 8: Sequences of Scoring Tiles

Tile ID	GENE	90-mer AA sequence
NM_016249-3.5	MAGE-C2	SEESSSQKGEDTGTCQGLPDSESSFTYTLDEKVAELVEFLLLKYE AEEPVTEAEMLMIVIKYKDYFPVILK***RAREFMELL***FGLALIEVGP
NM_016249-4.5	MAGE-C2	AEEPVTEAEMLMIVIKYKDYFPVILK***RAREFMELL***FGLALIEVGP DHFCVFANTVGLTDEGSDDEGMPENSLLIIILSVIFIKGNCASEE
NM_152385-9.5	CLHC1	LDLVTNWVTQERLTFSEEAGDVICDYGEQDTYNKAKCLALAQI VYSECGLHKKAILCLCKQGQTH**RVMEYIQQL**KDFTTDDLLQLL MSCP
NM_152385-10.B	CLHC1	CDYGEQDTYNKAKCLALAQIVYSECGLHKKAILCLCKQGQTH**R VMEYIQQL**KDFTTDDLLQLLMSCPQVELIQCLTKELNEKQPSLS FGL
NM_152385-10.5	CLHC1	SECGLHKKAILCLCKQGQTH**RVMEYIQQL**KDFTTDDLLQLLMS CPQVELIQCLTKELNEKQPSLSFGLAILHLFSADMKKVGIKLLQEI N
NM_134266-5.5	SLC26A7	MPYISGPLGFFYIYAYVFENIKSV**RLEALLLSLL**SIVVLVLVKELNE QFKRKIKVVLPVDLVLIIAASFACYCTNMENTYGLEVVGHIPQ
485053-REF46381_REF11532_SNP47179_SN P9358	CIT_TTN_PARP4_GC FC2	RTDDLKWSRLPLAFAYREPYLF|FCTKPITCKDELAPPTLHLDF**R |TKQLLGL**QPISSVSPLHRVLHY|KRELARAQDDYLSLDVQHTS SI
482327-REF39385_REF4416_SNP39624_SNP 1651	BRSK2_NBPF10_NLRP14_CELA3A	TYTEGGEAQKENGIYSVTFTLL|RRGRKEGEEDQNPPCPRLSRE L|ETQEDQEAVLGYGTEYRN**RIKE|WQVSL**QYEKSGGFYHTC GGSLI

Screening of the mechanistically representative TSC-201-B0702 TCR-T cells against the human proteome revealed putative off-targets of the therapeutic TCR: CLHC1 and SLC26A7. Consensus gene expression data from the Human Protein Atlas suggest that CLHC1 is ubiquitously expressed and SLC26A7 shows tissue-specific expression (summarized in Table 9). The two SNP clones express a bioinformatically predicted epitope

that falls in an artificial junction between two exons. This junction sequence has never been observed in normal cells. The putative off-targets would represent significant off-target reactivity risk if confirmed to represent bona fide off-targets of TSC-201-B0702.

Table 9: Summary of tissue specificity and function of the putative off-target proteins identified for TSC-201-B0702

Gene	Protein	Tissue Specificity	Summary
CLHC1	Clathrin heavy chain linker domain containing 1	Ubiquitous	Clathrin is a component of the cytoplasmic face of intracellular organelles involved in the intracellular trafficking of receptors and endocytosis of macromolecules (Royle *et. al.* (2006) *Cell Mol Life Sci.* 63:1823-1832).
SLC26A7	Solute carrier family 26 member 7	Thyroid	Members of the solute carrier family 26 transport anions in the epithelia of multiple organs (Dorwart *et al.* (2008)

Example 6: Alloreactivity profiling for TSC-201-B0702

The alloreactivity profile of the recombinant TCR used for TSC-201-B0702 TCR-T cells was evaludated. Alloreactivity is the ability of TCRs to recognize allogeneic MHC molecules that were not encountered during the thymic development and will potentially manifest itself clinically as manufacturing failure due to fratricide reactivity, and graft-versus-host disease (GvHD) upon infusion in patient. TSC-201-B0702 TCR-T cells display no alloreactivity to any of the 110 most common MHC alleles. This result predicts that TSC-201-B0702 will not present any adverse alloreactive responses in patients.

In this study, mechanistically representative TCR-T cells (*i.e.*, $CD3^+$ T cells engineered by lentivirus transduction to express the therapeutic TCR used in TSC-201-B0702 along with the CD8αβ co-receptor) were co-cultured for 48 hours with target cells (HEK293T cells) expressing individually each of the 110 most common class I MHCs in the US population (Maiers *et al.* (2007) *Erratum in: Hum Immunol.* 69:141) in an array format (refer to Table 10 for the complete list of MHC-I surveyed in this study and their frequency in the US population). The reactivity of the therapeutic TCR to allogeneic MHC molecules and the resulting cytotoxicity on target cells was assessed by measuring the target cell growth

inhibition caused by the TCR-T cells relative to untransduced (UTD) donor control $CD3^+$ T cells.

Mechanistically representative TSC-201-B0702 TCR-T cells elicited strong cell killing on HEK293T cells expressing HLA-B*07:02 and a 90 amino acid fragment containing the MAGE-C2 derived epitope, RAREFMELL, which served as a positive control for the killing assay. The co-culture of mechanistically representative TSC-201-B0702 TCR-T with the array found that the TCR-T cells had no significant reactivity to HEK293T cells expressing any of the 110 most frequent HLA alleles in the US population.

Table 10 List of the 110 MHCs surveyed in this study

HLA-A alleles	**freq.**	**HLA-B alleles**	**freq.**	**HLA-C alleles**	**freq.**
A*02:01:01:01	0.2375	B*07:02:01:01	0.1072	C*07:01:01:01	0.1410
A*01:01:01:01	0.1256	B*08:01:01:01	0.0902	C*07:02:01:01	0.1298
A*03:01:01:01	0.1149	B*44:02:01:01	0.0638	C*04:01:01:01	0.1275
A*24:02:01:01	0.0878	B*35:01:01:01	0.0587	C*06:02:01:01	0.0848
A*11:01:01:01	0.0551	B*44:03:01:01	0.0518	C*03:04:01:01	0.0752
A*23:01:01:01	0.0348	B*15:01:01:01	0.0486	C*05:01:01:01	0.0692
A*29:02:01:01	0.0330	B*51:01:01:01	0.0446	C*16:01:01:01	0.0466
A*68:01:01:01	0.0304	B*40:01:01	0.0432	C*02:02:01	0.0442
A*26:01:01:01	0.0273	B*18:01:01:01	0.0412	C*03:03:01:01	0.0435
A*32:01:01:01	0.0266	B*14:02:01:01	0.0293	C*12:03:01:01	0.0410
A*31:01:02:01	0.0260	B*57:01:01:01	0.0270	C*08:02:01:01	0.0382
A*30:01:01:01	0.0246	B*27:05:02:01	0.0240	C*01:02:01:01	0.0354
A*30:02:01:01	0.0209	B*53:01:01:01	0.0237	C*17:01:01:02	0.0204
A*68:02:01:01	0.0203	B*13:02:01:01	0.0209	C*15:02:01:01	0.0203
A*33:03:01:01	0.0162	B*38:01:01:01	0.0169	C*14:02:01:01	0.0142
A*25:01:01:01	0.0139	B*49:01:01:01	0.0169	C*12:02:01	0.0108
A*33:01:01:01	0.0129	B*40:02:01:01	0.0166	C*07:04:01:01	0.0104
A*02:06:01:01	0.0108	B*52:01:01:01	0.0150	C*08:01:01:01	0.0098
A*02:05:01:01	0.0107	B*58:01:01:01	0.0147	C*03:02:01	0.0077
A*74:01:01	0.0103	B*15:03:01:01	0.0139	C*18:01	0.0061
A*02:02:01:01	0.0088	B*35:03:01:01	0.0138	C*15:05:01:01	0.0061
A*34:02:01	0.0065	B*55:01:01:01	0.0132	C*16:02:01	0.0030
A*66:01:01:01	0.0050	B*45:01:01	0.0129	C*08:04:01	0.0023
A*36:01	0.0046	B*37:01:01:01	0.0109	C*03:05	0.0021
A*29:01:01:01	0.0029	B*42:01:01	0.0103	C*14:03	0.0015
A*02:07:01	0.0026	B*50:01:01:01	0.0093	**sum**	**0.9910**
A*03:02:01	0.0024	B*39:01:01:01	0.0089		
A*68:03:01	0.0024	B*35:02:01:01	0.0087		
A*02:03:01	0.0020	B*14:01:01:01	0.0083		
A*01:02	0.0016	B*58:02:01	0.0078		
sum	**0.9787**	B*57:03:01:01	0.0071		
		B*39:06:01	0.0068		

		B*15:10:01	0.0061		
		B*41:01:01:01	0.0053		
		B*48:01:01:01	0.0052		
		B*07:05:01:01	0.0046		
		B*56:01:01:01	0.0044		
		B*41:02:01:01	0.0043		
		B*39:05:01:01	0.0041		
		B*15:17:01:01	0.0040		
		B*35:08:01:01	0.0040		
		B*81:01:01	0.0038		
		B*15:16:01:01	0.0038		
		B*46:01:01	0.0037		
		B*35:12:01	0.0033		
		B*35:17:01	0.0028		
		B*40:06:01:01	0.0028		
		B*78:01:01:01	0.0025		
		B*15:02:01:01	0.0023		
		B*27:02:01:01	0.0023		
		B*38:02:01	0.0022		
		B*13:01:01:01	0.0021		
		B*47:01:01:02	0.0021		
		B*54:01:01	0.0020		
		B*15:18:01:01	0.0020		
		sum	**0.9660**		

Cell Lines

Cell Line	Vendor	Catalog Number
HEK293T	ATCC	CRL-3216
Lenti-X	Takara Bio	632180

Media and Supplements

Media	Vendor	Catalog Number
RPMI 1640	Gibco	A10491-01
DMEM	Gibco	11965
GlutaMAX supplement	Gibco	35050061
OptiPRO SFM	Gibco	12309019
Penicillin streptomycin	Gibco	15149-122
Heat-inactivated fetal bovine serum (FBS)	Gibco	A3840102

Reagents

Reagent	Vendor	Catalog
Recombinant human IL-2	PeproTech	AF-200-02
Recombinant human IL-7	R&D Systems	207-IL-025
Recombinant human IL-15	R&D Systems	247-ILB-005
Nourseothricin	GoldBio	N-500-1

APC anti-human HLA A,B,C Antibody	BioLegend	311410
CD3 monoclonal antibody (OKT3)	eBioscience	16-0037-81
ImmunoCult™ Human CD3/CD28/CD2 T cell activator	StemCell Technologies	10970
Incucyte® NucLight Red Lentivirus Reagent	Essen BioScience	4476
Mirus *Trans*IT®-293 Transfection Reagent	Mirus Bio	MIR2704
Alt-R® S.p. Cas9 Nuclease V3 (Lot #0000417827)	IDT	1081059
Alt-R® CRISPR-Cas9 tracrRNA (Lot #0000415438)	IDT	1072534
StraightFrom® Leukopak® CD3 Microbead Kit, human	Miltenyi Biotec	130-122-365
autoMACS® Running Buffer - MACS® Separation Buffer	Miltenyi Biotec	130-091-221
EasySep Buffer	StemCell Technologies	20144
CryoStor® CS10	StemCell Technologies	100-1061
Luna® Universal One-Step RT-qPCR Kit	New England BioLabs	E3005X
jetPRIME Transfection Reagent	Polyplus-transfection	114-75
SE Cell Line 4D-Nucleofector™ X Kit L	Lonza	V4XC-1012
CD34 Microbead Kit, human	Miltenyi Biotec	130-046-703

Lentiviral was packaged and the lentiviral titer was quantified. Lenti-X cells (Takara Bio USA, Mountain View, CA) were plated at 75% confluency and transfected using jetPRIME® transfection reagent (Polyplus, Illkirch, France). Briefly, lentiviral constructs were mixed with packaging plasmids (pREV/pTAT/pVSVG/pGAGPOL) and incubated with jetPRIME® reagent according to the manufacturer's protocol, and Opti-Pro™ SFM medium was added at 24 hours post-transfection. Viral supernatants were harvested 48 hours after transfection. TSC-201-B0702 viral supernatants were concentrated using either Vivaspin 20 centrifugal concentrators or Vivaflow 50 cassettes (Sartorius, Bohemia, NY). Virus titer was determined by RT-qPCR using Luna® Universal One-Step RT-qPCR Kit (New England BioLabs) on a QuantStudio™ 7 Pro (ThermoFisher Scientific). Briefly, virus was diluted in PBS, lysed and 2 µl of the lysed virus was used in RT-qPCR according to manufacturer's instructions. Standard curve was generated using serial diluted virus with known titer. Titer was expressed as TU/ml.

To generate mechanistically representative TSC-201-B0702 TCR-T cells, primary $CD3^+$ T cells from a single healthy donor were isolated using the StraightFrom® Leukopak® CD3 Microbead Kit (Miltenyi Biotec) according to the manufacturer's protocol. Isolated cells were frozen in CryoStor® CS10 (Stem Cell Technologies) and stored in liquid nitrogen until use. On Day -1, $CD3^+$ T cells were thawed, washed with complete T cell medium (RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum [FBS], 100 IU/mL penicillin, 100 µg/mL streptomycin, recombinant human IL-2 [50 U/mL, PeproTech, Cranbury, NJ], recombinant human IL-15 [5 ng/mL, R&D Systems], and recombinant human IL-7 [5 ng/mL, R&D Systems]), seeded at 1.0E6/mL, and rested overnight at 37°C. On Day

0, $CD3^+$ T cells were washed and resuspended in fresh T cell medium and activated using ImmunoCult™ human CD3/CD28/CD2 T cell activator (5 μL/1.0E6 $CD3^+$ T cells, Stem Cell Technologies). On Day 1, cells were washed and resuspended in fresh complete T cell medium and transduced with lentiviral particles to express the recombinant TCR for TSC-201-B0702.

On Day 2, cells were washed and resuspended in fresh complete T cell medium and expanded until Day 5 in G-Rex® 6-well plates (Wilson Wolf). On Day 5, cells were isolated using CD34 MicroBead kit (Miltenyi Biotec) according to the manufacturer's protocol. The isolated cells were resuspended in fresh complete T cell medium and expanded in G-Rex® 10 flasks (Wilson Wolf) until Day 12, at which point the cells were frozen down in CryoStor® CS10 and stored in liquid nitrogen until used.

A 96-well-based, MHC-expressing array was generated. Briefly, endogenous HLA-A/B/C were knocked out in HEK293T cells using CRISPR. Guide RNAs were designed against sequences conserved across the HLA-A, HLA-B and HLA-C loci using the multicrispr.net tool (Prykhozhij *et al.* (2015) Erratum in: PLoS One 10:e0138634). The following guides were selected: CRISPR-ALL-1: CGGCTACTACAACCAGAGCG, CRISPR-ALL-2: AGATCACACTGACCTGGCAG, CRISPR-ALL-3: AGGTCAGTGTGATCTCCGCA. gRNAs were cloned into the LentiCRISPR V2 vector using BsmBI sites. HEK293T cells were transfected with plasmid guide constructs using Mirus TransIT® (Mirus Bio, Madison, WI). After 7 days, MHC-negative cells were sorted using a pan-MHC antibody (BioLegend). Single cell clones were expanded, and the absence of MHC was verified by flow cytometry and western blot.

MHC-null HEK293T cells were transduced with IncuCyte® NucLight™ Red virus (Essen BioScience). Transduced wells were sorted for NucLight™ Red expression using a Sony SH800 sorter. To generate an MHC-expressing array, MHC-null NucLight™ Red-expressing HEK 293T cells were individually transduced with the most common 110 MHCs (pHAGE-EF1a-MHC-UBC-NAT) in individual wells in 96-well plates. Transduced cells were selected with nourseothricin (400 ng/ml, GoldBio) for one week. Cells expressing the most common 110 class I MHC alleles were passaged and stored in 96-well plates as an array.

To generate the positive control for the assay, the HLA-B*07:02-expressing cells in the above array were transduced with a 90 amino acid fragment which contains the MAGE-C2 epitope (RAREFMELL) and an AmCyan expression cassette. Cells were sorted for AmCyan expression using an Invitrogen™ Bigfoot™ Spectral Cell Sorter with Sasquatch

Software (SQS). Expression of individual MHC alleles in the array was verified by staining using a pan-MHC class I antibody (BioLegend).

Co-culture for alloreactivity profiling wes then set up. Specifically, on day 0, mechanistically representative TSC-201-B0702 TCR-T cells were restimulated in upright T25 flasks with 1E6 T cells, 20E6 irradiated PBMCs, recombinant human IL-2 [50 U/mL, Peprotech], and CD3 monoclonal antibody (OKT3) [0.1 μg/mL, eBioscience]. Half the volume of media was exchanged on days 2, 4, and 5 with RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum [FBS], 100 IU/mL penicillin, 100 μg/mL streptomycin, and recombinant human IL-2. On day 5, target cells in the 96-well array were passaged and seeded in 384-well plates. On day 6, T cells were harvested and resuspended in cytokine medium containing IL-2 [100U/mL] for the co-culture. The assay was performed in triplicate. Mechanistically representative TSC-201-B0702 TCR-T cells or untransduced control T cells were added at an effector:target (E:T) ratio of 5:1 and incubated with target cells for 48 hours. Target cell numbers were measured over time using an IncuCyte® S3. Cell growth inhibition at indicated timepoint of co-culture on each MHC-expressing HEK293T cells in the assay was calculated as 1-(Cell doubling[Incubated with mechanistically representative TSC-201-B0702 T cells]/Cell doubling[Incubated with UTD donor T cells]). To correct for batch effect of each 384-well plate, the cell inhibition value of each well (each MHC) on each 384-well plate were subtracted by the median of cell inhibition of the wells on each 384-well plate.

As shown in Figure 14, mechanistically representative TSC-201-B0702 TCR-T cells achieved complete (98.8 ± 0.002%) cell growth inhibition on the target cells expressing HLA-B*07:02 and the MAGE-C2 90mer containing the on-target epitope after 48 hour of co-culture. Conversely, no inhibition of target cell growth was observed when target cells lacking HLA expression were co-cultured with the TCR-T cells (see the blue condition in Figure 14), or when the TCR-T cells were co-cultured with target cells expressing HLA-B*07:02 but lacking MAGE-C2 expression (Figure 14). These conditions served as a positive and negative controls for the assay confirming that the effector T cells were functionally capable of reacting selectively to cells expressing their cognate peptide/MHC target.

In the absence of MAGE-C2 expression, TSC-201-B0702 TCR-T cells did not show significant inhibition of cell growth on target cells expressing any of the 110 most common class I MHC alleles. This result suggests that the therapeutic TCR used in the TSC-201-

B0702 TCR-T cells does not present any alloreactivity to the 110 MHC alleles surveyed (Figure 14).

Example 7: Lack of TSC-201-B0702 TCR-T cell off-tumor reactivity

The risk of on-target/off-tumor reactivity is low for TSC-201-B0702 TCR-T cells as MAGEC2 expression is largely restricted to the testis (Lucas *et al.* (2000) *Int. J. Cancer.* 87:55-60), which is an immune privileged site (Li *et al.* (2012) *Front Immunol.* 3:152). Data from the study illustrated in Example 8 below shows that MAGEC2 expression is largely restricted to testis with low MAGEC2 expression detected in the pancreas (~340-fold lower than testis), and pituitary glands (~80-fold lower than testis). These gene expression analyses indicate that MAGEC2 represents a safe target for T cell-based therapies with low risk for on-target off-tumor reactivity.

The off-target reactivity of a therapeutic TCR may arise from cross-reactivity of the TCR to a self-peptide/MHC. Genome-wide safety screen identified 2 putative off-targets of TSC-201-B0702 TCR-T cells: CLHC1 and SLC26A7 (see Example 5 above). As described below, TSC-201-B0702 TCR-T cells consistently showed no reactivity to any of the 54 primary and iPSC derived tissues tested, which represent physiologically relevant systems for testing off-target reactity of TCR-T cells. Moreover, no reactivity was observed to any of the 5 cancer cell lines tested that endogenously express putative off-targets. Collectively, these data indicate that none of the putative off-targets are bona fide off-targets of TSC-201-B0702. Thus, the reactivity of TSC-201-B0702 TCR-T cells is expected to be restricted to cancer cells expressing MAGE-C2 and HLA-B*07:02.

Generally, process-representative TSC-201-B0702 TCR-T cells produced from three independent donors were systematically tested for their reactivity to an extensive panel of 59 target cells comprising 5 cancer cell lines (Table 11) and 54 healthy human primary cells and iPSC-derived cells from multiple tissues and organs (Table 11).

Table 11: Target cancer cell line, primary cell and iPSC-derived cell descriptions

Target cancer cell line descriptions

Cell Line	Vendor	Catalog number	Culture Medium
OVCAR-3	ATCC	HTB-161	RPMI-1640 (ATCC 30-2001) + 20% HIFBS (Fisher Scientific A3840002) + 0.01 mg/mL bovine insulin (Sigma-Aldrich I0516-5ML) + 1X Pen/strep (Fisher Scientific 15149-122)
GOS-3	DSMZ	CRL-5885	DMEM (Fisher Scientific 11965-092) + 10% HIFBS (Fisher Scientific A3840002) + 1X Pen/Strep (Fisher Scientific 15149-122)
EBC-1	Creative Bioarray	CSC-C6336J	EMEM (ATCC 30-2003) + 10% FBS (Fisher Scientific A3840002) + 1X Pen/Strep (Fisher Scientific 15149-122)
A2058 NLR	ATCC	CRL-11147	DMEM (Fisher Scientific 11965-092) + 10% HIFBS (Fisher Scientific A3840002) + 1X Pen/Strep (Fisher Scientific 15149-122)
COLO-783	*DSMZ*	*ACC 257*	RPMI-1640 (ATCC 30-2001) + 10% HIFBS (Fisher Scientific A3840002) + 1X Pen/Strep (Fisher Scientific 15149-122)
CaSki	*ATCC*	*CRL-5935*	RPMI-1640 (ATCC 30-2001) + 10% HIFBS (Fisher Scientific A3840002) + 1X Pen/Strep (Fisher Scientific 15149-122)

A2058 NLR is A2058 cell line transduced with NucLight Red lentivirus to express red fluorescent protein mKate2

Target primary and iPSC-derived cell descriptions

Primary/iPS-derived cell type	Lot/batch number	Catalog number	Vendor	Culture Medium (catalog number)
Peripheral Blood Mononuclear Cells (PBMC)	**12429**		AllCells	X-VIVO15 (Lonza 04-418Q) + 5% Human serum (BIOIVT HUMANABSRMP-1) + 1X GlutaMax (FisherScientific #35050061) + 1X Penicillin/Streptomycin (Fisher Scientific 15149-122)
	888607014		DLS	
	110044327		DLS	
Human Umbilical Vein Endothelial Cells (HUVEC)	474**Z035**	A-12991	PromoCell	Endothelial Cell Growth Medium 2 (C-22011)
	474**Z036**	C-12206		
	486**Z026**	C-12200		Endothelial Cell Growth Medium (C-22010)
Normal Human Dermal Fibroblasts (NHDF)	477**Z039**.2	C-12300	PromoCell	Fibroblast Growth Medium (C-23010)
	473**Z011**.2	C-12302		Fibroblast Growth Medium 2 (C-23020)

Human Pulmonary Fibroblasts (HPF)	1081503.3 474Z031.2 3080702	C-12360	PromoCell	Fibroblast Growth Medium 2 (C-23020)
Human Cardiac Fibroblasts (HCF)	9601	6320	ScienCell	Fibroblast Medium-2 (2331)
Human Aortic Smooth Muscle Cells (HAoSMC)	437Z016.2 453Z011.6 454Z019.2	C-12533	PromoCell	Smooth Muscle Cell Growth Medium 2 (C-22062)
Human Small Airway Epithelial Cells (HSAEpC)	467Z033 435Z002 480Z008.2	C-12642	PromoCell	Small Airway Epithelial Cell Growth Medium (C-21070)
Human Bronchial Smooth Muscle Cells (HBSMC)	432Z016.3 458Z006.2	C-12561	PromoCell	Smooth Muscle Cell Growth Medium 2 (C-22062)
Normal Human Epidermal Melanocytes (NHEM)	473Z015.1	C-12400	PromoCell	Melanocyte Growth Medium (C-24010)
Human Cardiac Myocytes (HCM)	463Z016	C-12810	PromoCell	Myocyte Growth Medium (C-22070)
	0123W	CSC-C2847	Creative Bioarray	SuperCult Human Cardiomyocytes Growth Medium (CM-7863G)
Human White Preadipocytes-subcutaneous (HWP)	465Z008.3 454Z006	C-12735	PromoCell	Preadipocyte Growth Medium (C-27410) Preadipocyte Differentiation Medium (C-27436) Adipocyte Nutrition Medium (C-27438)
Astrocytes	21098	ABC-TC3782	Accegen	Astrocytes Medium (ABM-TM3969)
Hepatocytes	HH1052 HH1165 HH1153	82006	In Vitro ADMET Laboratories	UCRM (81015) and UPCM (81016)
	300079-1	10HU-210		

Human Hepatic Stellate Cells (HHSteC)	300075-8		iXCells Biotech	Stellate Cell Growth Medium (MD-0014)
Human Skeletal Muscle Cells (HSkMC)	**4820**	3500	ScienCell	Skeletal Muscle Cell Medium (3501)
	F121213W14	H-6167	Cell Biologics	Smooth Muscle Cell Medium /w Kit –500 ML (M2268)
Retinal Pigment Epithelial Cells (RPEC)	**23522**	6540	ScienCell	Epithelial Cell Medium (4101)
Human Prostate Epithelial Cells (HPrEpC)	0**6335**	FC-0038	Lifeline Cell Technology	ProstaLife basal medium (LM-0017) + ProstaLife LifeFactors kit (LS-1072)
	0**2321**			
iCell® Microglia, **01279**	106068	C1110	Fujifilm Cellular Dynamics	iCell Glial Basal Medium (M1034) + iCell Microglia Supplement A (M1036) +iCell Microglia Supplement B (M1037) +iCell Microglia Supplement C (M1035)
Human aortic endothelial cells (HAoEC)	482**Z007**.2	C-12271	PromoCell	Endothelial Cell Growth Medium MV (C-22020)
	473**Z030**.1			
Human pulmonary artery endothelial cells (HPAEC)	482**Z007**.13	C-12241	PromoCell	Endothelial Cell Growth Medium (C-22010)
	458**Z016**.15			
	483**Z028**.2			
Normal human epidermal keratinocytes (NHEK)	18TL25**5024**	00192627	Lonza	KGM™ Gold Keratinocyte Growth Medium BulletKit™ (00192060)
Human Ovarian Surface Epithelial Cells (HOSEpC)	**7859**	7310	ScienCell	Ovarian Epithelial Cell Medium (7311)
Human Bronchial Epithelial Cells (HBEpC)	20TL09**4987**	CC-2540	Lonza	BEGM™ Bronchial Epithelial Cell Growth Medium BulletKit™ CC-3170
	470**Z013**	C-12640	PromoCell	Airway Epithelial Cell growth Medium (C-21060)
	8963	4120	ScienCell	Epithelial Cell medium (4101)

Human Renal Epithelial Cells (HREpC)	19TL036410	CC-2556	Lonza	REGM Renal Epithelial Cell Growth Medium BulletKit (CC-3190)
Human CD34+ Progenitor Cells from Cord Blood (hCD34+-CB)	483Z016	C-12921	PromoCell	StemSpan SFEMII (StemCell Technologies 09655)
	487Z026	C-12921		
	494Z029	C-12921		
Megakaryocytes (differentiated from CD34+ progenitor cells from cord blood)	483Z016	C-12921	PromoCell	StemSpan SFEMII (StemCell Technologies 09655) + StemSpan™ Megakaryocyte Expansion Supplement (100X) (StemCell Technologies 02696)
	487Z026	C-12921		
	494Z029	C-12921		

Text in bold in the 'Lot/batch number' column is used to identify the lot/batch of the target cells in the graphs. PBMCs were isolated from whole Leukopaks purchased from the vendors indicated in the table.

T cell media

Media	Components	Stock (mL)	Catalog Number
"Cytokine-free T cell medium"	X-VIVO 15	1000	Lonza#04-418Q
	Human Serum (Heat-Inactivated)	50	BIOIVT# HUMANABSRMP-1
	Pen Strep	10	Gibco#15149-122
	GlutaMAX (100x)	10	FisherScientific#35050061
"T cell medium with Cytokines"	Cytokine-free T cell medium	500	NA
	Human IL-2 (5 µg/mL)	2.5	Sigma-Aldrich#11147528001
	Human IL-7 (10 µg/mL)	0.25	R&D Systems#207-IL-025

Media and supplements

Media	Vendor	Catalog Number
X-VIVO 15	Lonza	04-418Q
Media	Vendor	Catalog
Human Serum (Heat-Inactivated)	Sigma-Aldrich	H3667-100
FBS, heat-inactivated	BIOIVT	HUMANABS
GlutaMAX (100x)	Fisher Scientific	35050061
Penicillin-Streptomycin	Gibco	15140-122
RPMI medium 1640	Gibco	A10491-01
DMEM	Gibco	11965
EMEM medium	ATCC	30-2003
Endothelial Cell Growth Medium 2	PromoCell	C-22011

Endothelial Cell Growth Medium	PromoCell	C-22010
Endothelial Cell Growth Medium MV	PromoCell	C-22020
Fibroblast Growth Medium	PromoCell	C-23010
Fibroblast Growth Medium 2	PromoCell	C-23020
Fibroblast medium-2	ScienCell	2331
Smooth Muscle Cell Growth Medium 2	PromoCell	C-22062
Small Airway Epithelial Cell Growth Medium	PromoCell	C-21070
Airway Epithelial Cell Growth Medium	PromoCell	C-21060
BEGM™ Bronchial Epithelial Cell Growth	Lonza	CC-3170
Melanocyte Growth Medium	PromoCell	C-24010
KGM™-2 Keratinocyte Growth Medium-2	Lonza	CC-3107
REGM Renal Epithelial Cell Growth Medium	Lonza	CC-3190
Myocyte Growth Medium	PromoCell	C-22070
Preadipocyte Growth Medium	PromoCell	C-27410
Preadipocyte Differentiation Medium	PromoCell	C-27436
Adipocyte Nutrition Medium	PromoCell	C-27438
SuperCult Human Cardiomyocytes Growth	Creative Bioarray	CM-7863G
Human Astrocytes Medium Kit	Accegen	ABM-
Universal Cryopreserved Hepatocyte	In Vitro ADMET	81015
Universal Hepatocyte Primary Cell Plating	In Vitro ADMET	81016
Stellate Cell Growth Medium	iXCells Biotechnology	MD-0014
Skeletal Muscle Cell Medium	ScienCell	3501
Smooth Muscle Cell Medium	Cell Biologics	M2268
Epithelial Cell Medium	ScienCell	4101
ProstaLife basal medium	Lifeline Cell	LM-0017
ProstaLife LifeFactors kit	Lifeline Cell	LS-1072
iCell Microglia Media Kit	Fujifilm Cellular	R1204
StemSpan SFEM II	StemCell Technologies	09655
StemSpan™ Megakaryocyte Expansion	StemCell Technologies	02696
StemSpan CD34+ expansion supplement (10X)	StemCell Technologies	2691

Reagents

Reagent	**Vendor**	**Catalog Number**
DPBS without CaCl2, MgCl2	Thermofisher Scientific	14190-144
TrypLE	Thermofisher Scientific	1260-08
ViaStain AOPI Staining Solution	Nexcelom Bioscience	CS2-0106-25mL
DMSO	Sigma Life Science	D2650-100mL
Interleukin-2, recombinant human (rhIL-2)	Sigma-Aldrich	1147528001
Interleukin-7, recombinant human (rhIL-7)	R&D Systems	207-IL-025
Simple Plex Cartridge for 72 samples, 1 analyte Human -IFNg (3rd Gen)	Biotechne, Protein Simple	SPCKB-CS-002697

RNeasy Plus Mini Kit	Qiagen	74124
Tapestation RNA ScreenTape Ladder	Agilent	5067-5578
Tapestation RNA ScreenTape Sample Buffer	Agilent	5067-5577
Qubit RNA BR	ThermoFisher	Q10210
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	NEB	E7760S
SPRIselect	Beckman Coulter	B23319
Qubit dsDNA HS	ThermoFisher	Q32854
Tapestation High Sensitivity D5000 Reagents	Agilent	5067-5593
Tapestation High Sensitivity D5000 Ladder	Agilent	5067-5594
PhiX Control v3	Illumina	FC-110-3001
NextSeq 1000/2000 P1 Reagents (300 Cycles)	Illumina	20050264
NextSeq 1000/2000 P2 Reagents (200 Cycles) v3	Illumina	20046812
NextSeq 2000 P3 Reagents (300 Cycles)	Illumina	20040561
APC anti-human CD41 antibody	Biolegend	303709
PE anti-human CD42b antibody	Biolegend	303905
HLA-B7, B27 Antibody, anti-human, APC, REAfinity™	Miltenyi Biotec	130-123-554

IFN-γ levels were determined in the culture supernatants as a measure of T cell reactivity. Bulk RNA sequencing was performed on all target cells to determine the expression of the putative off-targets of TSC-201-B0702 TCR identified in Example 5 above. The process-representative TCR-T cells produced from three independent donors were systematically tested against each target cell.

Figure 15 outlines steps and timelines of the cytokine assay used to test off-tumor reactivity of TSC-201-B0702 TCR-T cells. Briefly, process-representative TSC-201-B0702 TCR-T cells and donor-matched UTF control T cells from three independent donors (as generated in Example 3 above) were co-cultured with HLA-B*07:02$^+$ primary and iPSC-derived human cells and cancer cell lines for 20-28 hours. Culture supernatants were collected and evaluated for IFN-γ levels as a measure of T cell reactivity to the target cells. The reactivity of TSC-201-B0702 to the target cells was compared to multiple positive and negative controls. Multiple negative controls were used to establish the baseline IFN-γ levels in the assay. These included (1) donor-matched UTF T cells co-cultured with the same target cells, (2) TSC-201-B0702 TCR-T cells and UTF T cells co-cultured with negative control cell line CaSki or A2058 NLR (A2058 cell line transduced with NucLight™ Red lentivirus to express red fluorescent protein mKate2), (3) TSC-201-B0702 and UTF T cells cultured alone in the absence of any targets, and (4) target cells cultured alone in the absence of any T cells. Positive controls were included to ensure that TSC-201-B0702 TCR-T cells and target cells used in the assay were functional. Target cells pulsed with the MAGE-C2-derived peptide

RAREFMELL were co-cultured with TSC-201-B0702 TCR-T cells to assess if the target cells were healthy and express sufficient levels of HLA to activate TCR-T cells in response to the cognate peptide/MHC (pMHC). TSC-201-B0702 TCR-T cells were co-cultured with positive control cell line COLO-783 to establish IFN-γ levels in response to endogenous MAGEC2 and HLA expression.

Target cells and TCR-T cells were thawed. Cancer cell lines were thawed in a 37°C water bath and washed once with their respective cell culture medium to remove cryopreservation reagents. Cells were subsequently resuspended in their respective cell culture medium and cultured following standard procedures in 75 cm^2 or 175 cm^2 flasks (adherent target cells) at 37°C and 5% CO_2. Cells were kept at a sub-confluent state, in the exponential growth phase and passaged once or twice a week as needed. The cancer cell lines were maintained in culture no longer than 5 weeks prior to the initiation of the co-culture with TCR-T cells.

Primary human cells and iPSC-derived cells were thawed and seeded in appropriate media (Table 11) in tissue culture flasks/plates as per the manufacturer's recommendations. Media was replaced on primary cells the day after thaw with appropriate fresh media. Media was replaced thereafter as per the manufacturer's recommendations until the cells reached confluency and were ready for co-culture with TCR-T cells. Human white preadipocytes (HWP) were differentiated to adipocytes for two weeks and then used as target cells in the co-culture with TCR-T cells. Human cardiac myocytes (HCM) were allowed to mature in culture for 2 weeks before testing them as targets in the co-culture. Peripheral blood mononuclear cells (PBMC) were used as targets immediately post-thaw. iCell Microglia were plated directly in 96-well plates post-thaw and used for co-culture after a week. Hepatocytes were thawed and plated directly in 96 well flat bottom plates at the vendor-recommended density. Human $CD34^+$ progenitor cells from cord blood ($hCD34^+$-CB) were expanded for a week prior to using them for co-culture. In addition, megakaryocytes were differentiated from $hCD34^+$-CB cells, stained for CD41 and CD42b using flow cytometry to confirm differentiation, and then tested as targets in co-culture.

TCR-T cells were thawed in a manner where on day -1, T cells were thawed in a 37°C water bath and washed with cytokine-free T cell medium to remove cryopreservation reagents prior to being resuspended in T cell medium (Table 11) containing cytokines. T cells were seeded in G-REX® 6-well plates at a density of 1E6-2E6 viable cells/mL and allowed to recover in a humidified incubator at 37°C and 5% CO_2 for ~20-28 hours.

Adherent target cancer cell lines were plated one day prior to setting up the co-culture (*i.e.*, day -1). Cells were resuspended in respective media at a density of 0.5E6 cells/mL. Target cells were plated in 100 µL of their respective medium (Table 11) in 96 well flat bottom plates at 50,000 cells per well and allowed to attach overnight in the 37°C incubator. Hepatocytes were thawed and plated directly in 96 well flat bottom plates at the vendor-recommended densities on day -1. Likewise, HWP, HCM and iCell Microglia were plated directly at vendor-recommended seeding densities.

Cancer cell lines and primary cells were separately collected in 96-well V-bottom plates (5E4-2E5 cells each) at the time of plating of the target cells, washed with EasySep buffer twice and stained with HLA-B7 antibody at 4°C for 20 mins. Following a wash with EasySep buffer, the cells were stained with DAPI and acquired on Cytoflex S. Cells positive for DAPI were excluded as dead cells and cells negative for DAPI were interrogated for HLA-B7 expression.

Cancer cell lines and primary cells were separately collected in tubes (2E5-3E6 cells each) and following a PBS wash, were flash frozen as pellets for RNA sequencing to determine expression of off-targets of the therapeutic TCR used in TSC-201-B0702 TCR-T cells.

Cancer cell lines and primary cells were separately collected in tubes (2E5-3E6 cells each) and following a PBS wash, were flash frozen as pellets for RNA sequencing to determine expression of off-targets of the therapeutic TCR in TSC-201-B0702 TCR-T cells. The RNASeq data for 2 cancer cell lines and 39 primary cell lots from appropriate passage numbers and timepoints were previously generated at TScan Therapeutics.

Co-culture was set up on Day 0. Suspension target cells were resuspended in respective media at a density of 1E6 cells/mL. Target cells were plated in 100 µL of their respective medium (Table 11) in 96 well flat bottom plates at 100,000 cells per well. MAGE-C2 peptide was diluted to a final concentration of 200 ng/mL in cytokine-free T cell medium. On the day of co-culture, cells from relevant wells were peptide-pulsed with 100 µL peptide-containing media for a final peptide concentration of 100 ng/mL and incubated for 2-3 hours at 37°C, 5% CO_2. Post incubation, cells were gently washed 3 times with cytokine-free T cell media and 100 µL of target cell media was added. The plates were observed under the microscope to confirm that the cells remained attached unless described otherwise. Suspension target cells were peptide-pulsed in tubes at a final concentration of 100 ng/mL for 2-3 hours, washed twice with cytokine-free T cell media, resuspended at a density of 1E6/mL

and plated in 100 µL of their respective medium (Table 11) in 96 well flat bottom plates at 100,000 cells per well.

TSC-201-B0702 TCR-T cells and donor-matched UTF control T cells were collected after overnight recovery, resuspended in cytokine-free T cell media and 100 µL of 80,000 $CD34^+/Q^+$ TSC-201-B0702 TCR-T cells were added to the appropriate wells for co-culture with target cells. The same total numbers of corresponding UTF control T cells were added for any given T cell donor. For the 'T cell only' control, 100 µL of TCR-T cell suspension was plated along with 100 µL of cytokine-free T cell media.

For the 'target cell only' wells, 100 µL of cytokine-free T cell media was added to target cell media. Co-cultures were incubated at 37°C, 5% CO_2 for 20-28 hours.

Ella-based assessment of TCR reactivity for their cognate pMHC was performed on day 1-8. IFN-γ secretion was measured to evaluate the reactivity of TCR-T cells and donor-matched UTF T cells to cancer cell lines, primary and iPSC-derived human cells. After 20-28 hours of co-culture at 37°C and 5% CO_2, supernatants were collected and frozen at -80°C. After thawing the supernatants, IFN-γ analysis was performed on the Protein Simple ELLA (automated ELISA platform) according to the manufacturer's instructions. The raw data were exported and graphed in GraphPad Prism (v9.4.1).

Bulk RNA sequencing was performed to determine expression of the putative off-targets of TSC-201-B0702 TCR-T cells. Total RNA was extracted from cell pellets using RNeasy® Plus Mini kits. Following manufacturer's instructions, poly-adenylated RNA was isolated from up to 1 µg total RNA using NEBNext® Poly(A) mRNA Magnetic Isolation Module and directional RNAseq libraries were constructed using NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina (NEB, E7760S). Libraries were then sequenced paired-end (100bp x 100bp) on an Illumina NextSeq®2000 machine. Fastq files were preprocessed prior to alignment and gene expression calculation. The tools used for the preprocessing were Trimmomatic to cut adapter and Illumina-specific sequences from the read. Alignment was performed using hisat2 and annotated to the GRCh38 reference. The GRCh38 reference was taken from Gencode, Release 30 (GRCh38.p12). The resulting alignment files were coordinate sorted using Samtools before gene expression calculation by subReads featureCounts function. Feature counts were converted to TPM (Transcript Per Million) by first dividing the read counts by the length of each gene in kilobases to get reads per kilobase (RPK). RPK is summed per sample then divided by 1,000,000 to give a "per million" scaling factor to generate final TPM for each sample. The color scale used in RNAseq heatmaps has TPM values of zero set to white and values above zero follow a

continuous color scale up to 100 TPM as per the legend next to each heatmap in Figures 16 and 18.

a. Primary cell expression of putative off-targets

Bulk RNA sequencing was performed on target cells to determine expression of MAGEC2 and the putative off-targets of TSC-201-B0702 identified in Example 5 above. As shown in Figure 16, varying levels of expression of CLHC1 were detected in the cell types tested in this study. Internal RNASeq data showed that out of all the primary cells tested, the highest CLHC1 expression of 13 TPM was observed in $hCD34^{+}$-CB cells. All other primary cell types exhibited CLHC1 expression ranging from 0.3 to 3 TPM. SLC26A7 was expressed at a TPM of less than 0.2 TPM in the panel of primary cells tested; therefore, a panel of cancer cell lines expressing SLC26A7 was included to test the off-target reactivity of TSC-201-B0702 TCR-T cells (Figure 18 and Figures 19A-19C). MAGEC2 expression is largely restricted to the testis; however, lower yet detectable levels are observed in the pancreas and pituitary gland by qPCR (see Example 8 below). Expression of MAGEC2 ranged from 0 to 0.7 TPM for all primary and iPSC-derived cells tested. These gene expression analyses coupled with initial safety data from clinical trials of TCR-T therapy targeting PRAME suggest an overall low risk for on-target, off-tumor reactivity. Altogether, these data indicate that the primary cell panel includes an extensive collection of cell types that endogenously express CLHC1, and any reactivity (or lack thereof) is informative for assessing risk of off-tumor reactivity.

b. Lack of TSC-201-B0702 TCR-T cell off-tumor reactivity to primary and iPSC-derived cells

The reactivity of TSC-201-B0702 was evaluated against a selected panel of 54 HLA-B*07:02-positive healthy primary human cells and iPSC-derived cells. This panel included cells from various lineages such as epithelial, mesenchymal, endothelial, fibroblastic, muscle cells derived from multiple vital and non-vital organs, reproductive and non-reproductive organs, derived from male and female donors comprising the organs/tissues that are traditionally assessed during toxicology studies.

As shown in Figure 16, the primary cell panel includes an extensive collection of cell types that endogenously express CLHC1, and any reactivity (or lack thereof) is informative for assessing risk of off-tumor reactivity.

Figures 17A-17C provide a representative graph demonstrating that no reactivity of three batches of process-representative TSC-201-B0702 TCR-T cells and donor-matched untransfected (UTF) control T cells (PD353, PD354, and PD355) co-cultured with (1) three lots of HLA-B*07:02-positive peripheral blood mononuclear cells (PBMCs) or (2) three lots of HLA-B*07:02-positive human $CD34^+$ progenitor cells from cord blood ($hCD34^+$-CBs), respectively, was observed. Similar data and results were obtained for a assays in which the batches of TSC-201-B0702 TCR-T cells and donor-matched untransfected (UTF) control T cells (PD353, PD354, and PD355) co-cultured with the other primary cell types. No reactivity of TSC-201-B0702 TCR-T cells was observed for any of the primary cell types tested in this study. In addition, the positive and negative controls performed as expected, demonstrating the validity of these assays (see, for example, Figures 17A-17C).

c. Cancer cell line expression of putative off-targets

To demonstrate safety and lack of off-target reactivity, TSC-201-B0702 TCR-T cells and donor-matched UTF control T cells were co-cultured with a selected panel of HLA-B*07:02 positive cancer cell lines comprising OVCAR-3, GOS-3, EBC-1, A2058 NLR and CaSki.

Briefly, RNASeq was performed on OVCAR-3, GOS-3, EBC-1, A2058 NLR and CaSki cell lines to confirm the expression of putative off-targets in cancer cell lines used to test the off-target reactivity of TSC-201-B0702 TCR-T cells. As shown in Figure 18, COLO-783 showed high MAGEC2 expression (538.7 TPM) and served as a positive control in this assay to demonstrate that the TCR-T cells are functional. CaSki had no MAGEC2 expression and served as a negative control target cell line in this assay. A2058 NLR expressed MAGEC2 at 23.9 TPM while OVCAR-3, GOS-3 and EBC-1 expressed MAGEC2 at less than 0.5 TPM. However, no reactivity of TSC-201-B0702 TCR-T cells was observed to these cell lines (Figures 19A-19C). CLHC1 expression ranged from 0.2 to 5 TPM, with GOS-3 showing the highest expression of 5 TPM followed by OVCAR-3 at 2.6 TPM and EBC-1 at 1.4 TPM. CaSki and A2058 NLR expressed lower than 1 TPM of CLHC1. SLC26A7 expression ranged from 0.02 TPM to 27.3 TPM, with OVCAR-3 showing the highest expression of 27.3 TPM, followed by GOS-3 at 22.2 TPM. EBC-1, CaSki and A2058 NLR expressed lower than 2 TPM of SLC26A7. These data indicate that the cancer cell lines selected for this study naturally express all putative off-targets of TSC-201-B0702 TCR-T cells and are informative to assess the risk of off-target reactivity of TSC-201-B0702 TCR-T cells.

d. Lack of TSC-201-B0702 TCR-T cell reactivity to putative off-targets

To evaluate reactivity of TSC-201-B0702 TCR-T cells to putative off-targets, 3 batches of process-representative materials (PD353, PD354, and PD355) were co-cultured with OVCAR-3, GOS-3, EBC-1, A2058 NLR and CaSki cell lines and IFN-γ levels were determined in the culture supernatants as a measure of T cell reactivity.

As shown in Figures 19A-19C, the negative and positive controls performed as expected demonstrating the validity of the assay. All batches of TSC-201-B0702 TCR-T cells secreted IFN-γ in response to peptide-pulsed OVCAR-3, GOS-3, EBC-1 and CaSki cells. A2058 NLR expressed the lowest amount of HLA-B*07:02 among all the cell lines and induced no to low secretion of IFN-γ upon peptide-pulse from TCR-T cells. In the absence of exogenous peptide-pulse, baseline levels of IFN-γ were observed for all batches of TSC-201-B0702 TCR-T cells similar to those observed for control UTF T cells indicating lack of reactivity of the therapeutic T cells to the cancer cell lines tested. Altogether, these cancer cell lines express CLHC1 and SLC26A7 indicating lack of reactivity of TCR-T cells to physiological levels of these proteins.

Example 8: MAGE-C2 target expression in normal human tissues

As described above, MAGE-C2 (Melanoma-associated antigen C2) is a cancer /testis protein that is virtually absent in all normal adult tissues except spermatocytes (Lucas *et al.* (2000) *Int. J. Cancer.* 87:55-60), which is an immune privileged site (Li *et al.* (2012) *Front Immunol.* 3:152). To confirm that MAGE-C2 expression is enriched in testes, but not other normal tissues, cDNA arrays were purchased from a commercial vendor containing cDNA from 48 different normal (non-cancer) tissue sections and an additional 24 different subsection of normal (non-cancer) tissue sections and assayed for MAGE-C2 and GAPDH gene expression. As described below, MAGE-C2 expression in normal tissues is largely restricted to the testes, which, coupled with the same conclusions from prior literature and public gene expression databases, indicates that MAGE-C2 is a safe target for T cell-based therapies with low risk for on-target off-tumor reactivity for TSC-201-B0702.

Specifically, cDNA arrays in 96-well plate format were purchased from Origene and handled according to manufacturer instructions. A master mix consisting of TaqMan® Fast Advanced Master mix (ThermoFisher, cat # 4444557), Nuclease-free water (Invitrogen, cat # AM9937), MAGE-C2 and GAPDH TaqMan probes, were aliquoted into the 96-well cDNA array plate, 20 µL per well. Gene expression was measured on a QuantStudio 7 and Cq

values were quantified, analyzed, and graphed for the normal tissue cDNA array (Origene, cat #HMRT104).

Table 12 Materials

Material	Vendor	Catalog Number
Nuclease-Free Water	Invitrogen	AM9937
GAPDH (VIC) Taqman Probe	ThermoFisher Scientific	Hs02786624_g1
MAGE-C2 (FAM) Taqman Probe	Integrated DNA Technologies	Hs.PT.58.23276508
TaqMan Fast Advanced Master Mix	ThermoFisher Scientific	4444557
TissueScan- Human Normal cDNA Array	OriGene	HMRT104

Cq values were acquired from the QuantStudio 7 Pro for the TissueScan cDNA array. Cq values for MAGE-C2 was normalized to GAPDH to obtain the delta CT (ΔCt). The ΔCt values were then quantified using the 2^-ΔCt method to indicate levels of MAGE-C2 expression relative to levels of GAPDH.

Figure 20 shows MAGE-C2 expression normalized to GAPDH expression across 48 tissue types measured from the Origene TissueScan Human normal cDNA array. In agreement with publicly available gene expression databases, of the 48 normal tissues tested, only testes exhibited evident MAGE-C2 expression.

Incorporation by Reference

All publications, patents, and patent applications mentioned herein are hereby incorporated by reference in their entirety as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

Also incorporated by reference in their entirety are any polynucleotide and polypeptide sequences which reference an accession number correlating to an entry in a public database, such as those maintained by The Institute for Genomic Research (TIGR) on the World Wide Web at tigr.org and/or the National Center for Biotechnology Information (NCBI) on the World Wide Web at ncbi.nlm.nih.gov.

Equivalents and Scope

The details of one or more embodiments encompassed by the present invention are set forth in the description above. Although representative, exemplary materials and methods

have been described above, any materials and methods similar or equivalent to those described herein may be used in the practice or testing of embodiments encompassed by the present invention. Other features, objects and advantages related to the present invention are apparent from the description. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the present invention belongs. In the case of conflict, the present description provided above will control.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments encompassed by the present invention described herein. The scope encompassed by the present invention is not intended to be limited to the description provided herein and such equivalents are intended to be encompassed by the appended claims.

It is also noted that the term "comprising" is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term "comprising" is used herein, the term "consisting of" is thus also encompassed and disclosed.

Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges may assume any specific value or subrange within the stated ranges in different embodiments encompassed by the present invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

In addition, it is to be understood that any particular embodiment encompassed by the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions encompassed by the present invention (*e.g.*, any antibiotic, therapeutic or active ingredient; any method of production; any method of use; etc.) may be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

It is to be understood that the words which have been used are words of description rather than limitation, and that changes may be made within the purview of the appended claims without departing from the true scope and spirit encompassed by the present invention in its broader aspects.

While the present invention has been described at some length and with some particularity with respect to several described embodiments, it is not intended that it should be limited to any such particulars or embodiments or any particular embodiment, but it is to be construed with references to the appended claims so as to provide the broadest possible interpretation of such claims in view of the prior art and, therefore, to effectively encompass the intended scope encompassed by the present invention.

What is claimed is:

1. An immunogenic peptide comprising a peptide epitope selected from peptide sequences listed in Table 1.

2. An immunogenic peptide consisting of a peptide epitope selected from peptide sequences listed in Table 1.

3. The immunogenic peptide of claim 1 or 2, wherein the immunogenic peptide is derived from a MAGEC2 protein, optionally wherein the immunogenic peptide is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length.

4. The immunogenic peptide of any one of claims 1-3, wherein the immunogenic peptide is capable of eliciting an immune response against MAGEC2 and/or MAGEC2-expressing cells in a subject, optionally wherein the immune response is i) a T cell response and/or a CD8+ T cell response and/or ii) selected from the group consisting of T cell expansion, cytokine release, and/or cytotoxic killing.

5. An immunogenic composition comprising at least one immunogenic peptide according to any one of claims 1-4.

6. The immunogenic composition of claim 5, further comprising an adjuvant.

7. The immunogenic composition of claim 5 or 6, wherein the immunogenic composition is capable of eliciting an immune response against MAGEC2 and/or MAGEC2-expressing cells in a subject, optionally wherein the immune response is i) a T cell response and/or a CD8+ T cell response and/or ii) selected from the group consisting of T cell expansion, cytokine release, and/or cytotoxic killing.

8. A composition comprising a peptide epitope selected from peptide sequences listed in Table 1, and an MHC molecule.

9. The composition of claim 8, wherein the MHC molecule is an MHC multimer, optionally wherein the MHC multimer is a tetramer.

10. The composition of claim 8 or 9, wherein the MHC molecule is an MHC class I molecule.

11. The composition of any one of claims 9-10, wherein the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702.

12. A stable MHC-peptide complex, comprising an immunogenic peptide according to any one of claims 1-4 in the context of an MHC molecule.

13. The stable MHC-peptide complex of claim 12, wherein the MHC molecule is an MHC multimer, optionally wherein the MHC multimer is a tetramer.

14. The stable MHC-peptide complex of claim 12 or 13, wherein the MHC molecule is an MHC class I molecule.

15. The stable MHC-peptide complex of any one of claims 12-14, wherein the MHC molecule comprises an MHC alpha chain that is an HLA serotype of HLA-B*07, optionally wherein the HLA allele is HLA-B*0702.

16. The stable MHC-peptide complex of any one of claims 12-15, wherein the peptide epitope and the MHC molecule are covalently linked and/or wherein the alpha and beta chains of the MHC molecule are covalently linked.

17. The stable MHC-peptide complex of any one of claims 12-16, wherein the stable MHC-peptide complex comprises a detectable label, optionally wherein the detectable label is a fluorophore.

18. An immunogenic composition comprising the stable MHC-peptide complex according to any one of claims 12-17, and an adjuvant.

19. An isolated nucleic acid that encodes the immunogenic peptide of according to any one of claims 1-4, or a complement thereof.

20. A vector comprising the isolated nucleic acid of claim 19.

21. A cell that a) comprises the isolated nucleic acid of claim 19, b) comprises the vector of claim 20, and/or c) produces one or more immunogenic peptides according to any one of

claims 1-4 and/or presents at the cell surface one or more stable MHC-peptide complexes according to any one of claims 12-17, optionally wherein the cell is genetically engineered.

22. A device or kit comprising a) one or more immunogenic peptides according to any one of claims 1-4 and/or b) one or more stable MHC-peptide complexes according to any one of claims 12-17, said device or kit optionally comprising a reagent to detect binding of a) and/or b) to a binding protein, optionally wherein the binding protein is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain.

23. A method of detecting T cells that bind a stable MHC-peptide complex comprising:

a) contacting a sample comprising T cells with a stable MHC-peptide complex according to any one of claims 12-17; and

b) detecting binding of T cells to the stable MHC-peptide complex, optionally further determining the percentage of stable MHC-peptide-specific T cells that bind to the stable MHC-peptide complex, optionally wherein the sample comprises peripheral blood mononuclear cells (PBMCs).

24. The method of claim 23, wherein the T cells are CD8+ T cells.

25. The method of any one of claims 22-24, wherein the detecting and/or determining is performed using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

26. The method of any one of claims 22-25, wherein the sample comprises T cells contacted with, or suspected of having been contacted with, one or more MAGEC2 proteins or fragments thereof.

27. A method of determining whether a T cell has had exposure to MAGEC2 comprising:

a) incubating a cell population comprising T cells with an immunogenic peptide according to any one of claims 1-4 or a stable MHC-peptide complex according to any one of claims 12-17; and

b) detecting the presence or level of reactivity,

wherein the presence of or a higher level of reactivity compared to a control level indicates that the T cell has had exposure to MAGEC2, optionally wherein the cell population comprising T cells is obtained from a subject.

28. A method for predicting the clinical outcome of a subject afflicted with a disorder characterized by MAGEC2 expression comprising:

a) determining the presence or level of reactivity between T cells obtained from the subject and one more immunogenic peptides according to any one of claims 1-4 or one or more stable MHC-peptide complexes according to any one of claims 12-17; and

b) comparing the presence or level of reactivity to that from a control, wherein the control is obtained from a subject having a good clinical outcome,

wherein the presence or a higher level of reactivity in the subject sample as compared to the control indicates that the subject has a good clinical outcome.

29. A method of assessing the efficacy of a therapy for a disorder characterized by MAGEC2 expression comprising:

a) determining the presence or level of reactivity between T cells obtained from the subject and one more immunogenic peptides according to any one of claims 1-4 or one or more stable MHC-peptide complexes according to any one of claims 12-17, in a first sample obtained from the subject prior to providing at least a portion of the therapy to the subject, and

b) determining the presence or level of reactivity between the one more immunogenic peptides according to any one of claims 1-4, or the one or more stable MHC-peptide complexes according to any one of claims 12-17, and T cells obtained from the subject present in a second sample obtained from the subject following provision of the therapy to the subject,

wherein the presence or a higher level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the disorder characterized by MAGEC2 expression in the subject.

30. The method of any one of claims 27-29, wherein the level of reactivity is indicated by a) the presence of binding and/or b) T cell activation and/or effector function, optionally wherein the T cell activation or effector function is T cell proliferation, killing, or cytokine release.

31. The method of any one of claims 27-30, further comprising repeating steps a) and b) at a subsequent point in time, optionally wherein the subject has undergone treatment to ameliorate the disorder characterized by MAGEC2 expression between the first point in time and the subsequent point in time.

32. The method of any one of claims 27-31, wherein the T cell binding, activation, and/or effector function is detected using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

33. The method of any one of claims 27-32, wherein the control level is a reference number.

34. The method of any one of claims 27-33, wherein the control level is a level of a subject without the disorder characterized by MAGEC2 expression.

35. A method of preventing and/or treating a disorder characterized by MAGEC2 expression in a subject comprising administering to the subject a therapeutically effective amount of a composition according to any one of claims 1-22.

36. A method of identifying a peptide-binding molecule, or antigen-binding fragment thereof, that binds to a peptide epitope selected from the peptide sequences listed in Table 1 comprising:

a) providing a cell presenting a peptide epitope selected from the peptide sequences listed in Table 1 in the context of an MHC molecule on the surface of the cell;

b) determining binding of a plurality of candidate peptide-binding molecules or antigen-binding fragments thereof to the peptide epitope in the context of the MHC molecule on the cell; and

c) identifying one or more peptide-binding molecules or antigen-binding fragments thereof that bind to the peptide epitope in the context of the MHC molecule.

37. The method of claim 36, wherein the step a) comprises contacting the MHC molecule on the surface of the cell with a peptide epitope selected from the peptide sequences listed in Table 1.

38. The method of claim 36, wherein the step a) comprises expressing the peptide epitope selected from the peptide sequences listed in Table 1 in the cell using a vector comprising a heterologous sequence encoding the peptide epitope.

39. A method of identifying a peptide-binding molecule or antigen-binding fragment thereof that binds to a peptide epitope selected from the peptide sequences listed in Table 1 comprising:

a) providing a peptide epitope either alone or in a stable MHC-peptide complex, comprising a peptide epitope selected from the peptide sequences listed in Table 1, either alone or in the context of an MHC molecule;

b) determining binding of a plurality of candidate peptide-binding molecules or antigen-binding fragments thereof to the peptide or stable MHC-peptide complex; and

c) identifying one or more peptide-binding molecules or antigen-binding fragments thereof that bind to the peptide epitope or the stable MHC-peptide complex, optionally wherein the MHC or MHC-peptide complex is as according to any one of claims 8-17.

40. The method of claim 39, wherein the plurality of candidate peptide binding molecules comprises an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain.

41. The method of claim 39 or 40, wherein the plurality of candidate peptide binding molecules comprises at least 2, 5, 10, 100, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, or more, different candidate peptide binding molecules.

42. The method of any one of claims 39-41, wherein the plurality of candidate peptide binding molecules comprises one or more candidate peptide binding molecules that are obtained from a sample from a subject or a population of subjects; or the plurality of candidate peptide binding molecules comprises one or more candidate peptide binding molecules that comprise mutations in a parent scaffold peptide binding molecule obtained from a sample from a subject.

43. The method of claim 42, wherein the subject or population of subjects are a) not afflicted with a disorder characterized by MAGEC2 expression and/or have recovered from a

disorder characterized by MAGEC2 expression, or b) are afflicted with a disorder characterized by MAGEC2 expression.

44. The method of claim 42 or 43, wherein the subject or population of subjects has been administered a composition according to any one of claims 1-22.

45. The method of any one of claims 42-44, wherein the subject is an animal model of a disorder characterized by MAGEC2 expression and/or a mammal, optionally wherein the mammal is a human, a primate, or a rodent.

46. The method of any one of claims 42-45, wherein the subject is an animal model of a disorder characterized by MAGEC2 expression, an HLA-transgenic mouse, and/or a human TCR transgenic mouse.

47. The method of any one of claims 42-46, wherein the sample comprises peripheral blood mononuclear cells (PBMCs), T cells, and/or CD8+ memory T cells.

48. The peptide-binding molecule or antigen-binding fragment thereof identified according to any one of claims 39-47, optionally wherein the peptide-binding molecule or antigen-binding fragment thereof is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain.

49. A method of treating a disorder characterized by MAGEC2 expression in a subject comprising administering to the subject a therapeutically effective amount of genetically engineered T cells that express a peptide-binding molecule or antigen-binding fragment thereof that i) binds to a peptide epitope selected from the sequences listed in Table 1, ii) is identified according to the method according to any one of claims 39-48, and/or iii) binds to a stable MHC-peptide complex comprising a peptide epitopes selected from the sequences listed in Table 1 in the context of an MHC molecule, optionally wherein the peptide-binding molecule or antigen-binding fragment thereof is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain, optionally wherein the MHC or MHC-peptide complex is as according to any one of claims 8-17.

50. The method of claim 49, wherein the T cells are isolated from a) the subject, b) a donor not afflicted with the disorder characterized by MAGEC2 expression, or c) a donor recovered from a disorder characterized by MAGEC2 expression.

51. A method of treating a disorder characterized by MAGEC2 expression in a subject comprising transfusing antigen-specific T cells to the subject, wherein the antigen-specific T cells are generated by:

a) stimulating immune cells from a subject with a composition according to any one of claims 1-22; and

b) expanding antigen-specific T cells *in vitro* or *ex vivo*, optionally i) isolating immune cells from the subject before stimulating the immune cells and/or ii) wherein the immune cells comprise PBMCs, T cells, CD8+ T cells, naive T cells, central memory T cells, and/or effector memory T cells.

52. The method of claim 51, wherein the agents are placed in contact under conditions and for a time suitable for the formation of at least one immune complex between the peptide epitope, immunogenic peptide, stable MHC-peptide complex, T cell receptor, and/or immune cells.

53. The method of claim 51 or 52, wherein the peptide epitope, immunogenic peptide, stable MHC-peptide complex, and/or T cell receptor are expressed by cells and the cells are expanded and/or isolated during one or more steps.

54. The method of any one of claims 23-53, wherein the disorder characterized by MAGEC2 expression is a cancer or relapse thereof, optionally wherein the cancer is selected from the group consisting of melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma.

55. The method of any one of claims 23-54, wherein the subject is an animal model of a disorder characterized by MAGEC2 expression and/or a mammal, optionally wherein the mammal is a human, a primate, or a rodent.

56. A binding protein that binds a polypeptide comprising an immunogenic peptide sequence according to any one of claims 1-4, an immunogenic peptide according to any one of claims 1-4, and/or the stable MHC-peptide complex according to any one of claims 12-17,

optionally wherein the binding protein is an antibody, an antigen-binding fragment of an antibody, a TCR, an antigen-binding fragment of a TCR, a single chain TCR (scTCR), a chimeric antigen receptor (CAR), or a fusion protein comprising a TCR and an effector domain.

57. The binding protein of claim 56 comprising:

a) a T cell receptor (TCR) alpha chain CDR sequence with at least about 80% identity to a TCR alpha chain CDR sequence selected from the group consisting of TCR alpha chain CDR sequences listed in Table 2; and/or

b) a TCR beta chain CDR sequence with at least about 80% identity to a TCR beta chain CDR sequence selected from the group consisting of TCR beta chain CDR sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M.

58. The binding protein of claim 56 comprising:

a) a TCR alpha chain variable (V_α) domain sequence with at least about 80% identity to a TCR V_α domain sequence selected from the group consisting of TCR V_α domain sequences listed in Table 2; and/or

b) a TCR beta chain variable (V_β) domain sequence with at least about 80% identity to a TCR V_β domain sequence selected from the group consisting of TCR V_β domain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M.

59. The binding protein of claim 56 comprising:

a) a TCR alpha chain sequence with at least about 80% identity to a TCR alpha chain sequence selected from the group consisting of TCR alpha chain sequences listed in Table 2; and/or

b) a TCR beta chain sequence with at least about 80% identity to a TCR beta chain sequence selected from the group consisting of TCR beta chain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC

(pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M.

60. The binding protein of claim 56 comprising:

a) a TCR alpha chain CDR sequence selected from the group consisting of TCR alpha chain CDR sequences listed in Table 2; and/or

b) a TCR beta chain CDR sequence selected from the group consisting of TCR beta chain CDR sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M.

61. The binding protein of claim 56 comprising:

a) a TCR alpha chain variable (V_α) domain sequence selected from the group consisting of TCR V_α domain sequences listed in Table 2; and/or

b) a TCR beta chain variable (V_β) domain sequence selected from the group consisting of TCR V_β domain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M.

62. The binding protein of claim 56 comprising:

a) a TCR alpha chain sequence selected from the group consisting of TCR alpha chain sequences listed in Table 2; and/or

b) a TCR beta chain sequence selected from the group consisting of TCR beta chain sequences listed in Table 2, wherein the binding protein is capable of binding to a MAGEC2 immunogenic peptide-MHC (pMHC) complex, optionally wherein the binding affinity has a K_d less than or equal to about $5x10^{-4}$ M.

63. The binding protein of any one of claims 56-62, wherein 1) the TCR alpha chain CDR, TCR V_α domain, and/or TCR alpha chain is encoded by a TRAV, TRAJ, and/or TRAC gene or fragment thereof selected from the group of TRAV, TRAJ, and TRAC genes listed in Table 2, and/or 2) the TCR beta chain CDR, TCR V_β domain, and/or TCR beta chain is encoded by a TRBV, TRBJ, and/or TRBC gene or fragment thereof selected from the group of TRBV, TRBJ, and TRBC genes listed in Table 2, and/or 3) each CDR of the binding

protein has up to five amino acid substitutions, insertions, deletions, or a combination thereof as compared to the cognate reference CDR sequence listed in Table 2.

64. The binding protein of any one of claims 56-63, wherein the binding protein is chimeric, humanized, or human.

65. The binding protein of any one of claims 56-64, wherein the binding protein comprises a binding domain having a transmembrane domain, and an effector domain that is intracellular.

66. The binding protein of any one of claims 56-65, wherein the TCR alpha chain and the TCR beta chain are covalently linked, optionally wherein the TCR alpha chain and the TCR beta chain are covalently linked through a linker peptide.

67. The binding protein of any one of claims 56-66, wherein the TCR alpha chain and/or the TCR beta chain are covalently linked to a moiety, optionally wherein the covalently linked moiety comprises an affinity tag or a label.

68. The binding protein of claim 67, wherein the affinity tag is selected from the group consisting of aCD34 enrichment tag, glutathione-S-transferase (GST), calmodulin binding protein (CBP), protein C tag, Myc tag, HaloTag, HA tag, Flag tag, His tag, biotin tag, and V5 tag, and/or wherein the label is a fluorescent protein.

69. The binding protein of any one of claims 56-68, wherein the covalently linked moiety is selected from the group consisting of an inflammatory agent, cytokine, toxin, cytotoxic molecule, radioactive isotope, or antibody or antigen-binding fragment thereof.

70. The binding protein of any one of claims 56-69, wherein the binding protein binds to the pMHC complex on a cell surface.

71. The binding protein of any one of claims 56-70, wherein the MHC or MHC-peptide complex is as according to any one of claims 8-17.

72. The binding protein of any one of claims 56-71, wherein binding of the binding protein to the MAGEC2 peptide-MHC (pMHC) complex elicits an immune response, optionally wherein the immune response is i) a T cell response and/or a CD8+ T cell response

and/or ii) selected from the group consisting of T cell expansion, cytokine release, and/or cytotoxic killing.

73. The binding protein of any one of claims 56-72, wherein the binding protein is capable of specifically and/or selectively binding to the MAGEC2 immunogenic peptide-MHC (pMHC) complex with a K_d less than or equal to about $1x10^{-4}$ M, less than or equal to about $5x10^{-5}$ M, less than or equal to about $1x10^{-5}$ M, less than or equal to about $5x10^{-6}$ M, less than or equal to about $1x10^{-6}$ M, less than or equal to about $5x10^{-7}$ M, less than or equal to about $1x10^{-7}$ M, less than or equal to about $5x10^{-8}$ M, less than or equal to about $1x10^{-8}$ M, less than or equal to about $5x10^{-9}$ M, less than or equal to about $1x10^{-9}$ M, less than or equal to about $5x10^{-10}$ M, less than or equal to about $1x10^{-10}$ M, less than or equal to about $5x10^{-11}$ M, less than or equal to about $1x10^{-11}$ M, less than or equal to about $5x10^{-12}$ M, or less than or equal to about $1x10^{-12}$ M.

74. The binding protein of any one of claims 56-73, wherein the binding protein has a higher binding affinity to the peptide-MHC (pMHC) than does a known T-cell receptor, optionally wherein the higher binding affinity is at least 1.05-fold higher.

75. The binding protein of any one of claims 56-74, wherein the binding protein induces higher T cell expansion, cytokine release, and/or cytotoxic killing than does a known T-cell receptor when contacted with target cells with a heterozygous expression of MAGEC2, optionally wherein the induction is at least 1.05-fold higher.

76. The binding protein of claim 75, wherein the cytotoxic killing is a target cancer cell.

77. The binding protein of claim 76, wherein the cancer is selected from the group consisting of melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma.

78. The binding protein of any one of claims 56-77, wherein the binding protein does not bind to a pMHC complex comprising a CLHC1 and/or SLC26A7 peptide epitope.

79. A TCR alpha chain and/or beta chain selected from the group consisting of TCR alpha chain and beta chain sequences listed in Table 2.

80. An isolated nucleic acid molecule i) that hybridizes, under stringent conditions, with the complement of a nucleic acid encoding a polypeptide selected from the group consisting of polypeptide sequences listed in Table 2, ii) a sequence with at least about 80% homology to a nucleic acid encoding a polypeptide selected from the group consisting of the polypeptide sequences listed in Table 2, and/or iii) ii) a sequence with at least about 80% homology to a nucleic acid encoding listed in Table 2, optionally wherein the isolated nucleic acid molecule comprises 1) a TRAV, TRAJ, and/or TRAC gene or fragment thereof selected from the group of TRAV, TRAJ, and TRAC genes listed in Table 2 and/or 2) a TRBV, TRBJ, and/or TRBC gene or fragment thereof selected from the group of TRBV, TRBJ, and TRBC genes listed in Table 2.

81. The isolated nucleic acid of claim of claim 80, wherein the nucleic acid is codon optimized for expression in a host cell.

82. A vector comprising the isolated nucleic acid of claim 80 or 81, optionally wherein i) the vector is a cloning vector, expression vector, or viral vector and/or ii) the vector comprises a vector sequence listed in Table 3.

83. The vector of claim 82, wherein the vector further comprises a nucleic acid sequence encoding CD8α, CD8β, a dominant negative TGFβ receptor II (DN-TGFβRII), selectable protein marker, optionally wherein the selectable protein marker is dihydrofolate reductase (DHFR).

84. The vector of claim 83, wherein the nucleic acid sequence encoding CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker is operably linked to a nucleic acid encoding a tag.

85. The vector of claim 83 or 84, wherein the nucleic acid encoding a tag is at the 5' upstream of the nucleic acid sequence encoding CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker such that the tag is fused to the N-terminus of CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker.

86. The vector of claim 84 or 85, wherein the tag is a CD34 enrichment tag.

87. The nucleic acid or vector of any one of claims 80-86, wherein the nucleic acid sequence encoding TCRα, TCRβ, CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker are interconnected with an internal ribosome entry site or a nucleic acid sequence encoding a self-cleaving peptide.

88. The nucleic acid or vector of claim 87, wherein the self-cleaving peptide is P2A, E2A, F2A or T2A.

89. A host cell which comprises the isolated nucleic acid of claim 80 or 81, comprises the vector according to any one of claims 82-88, and/or expresses the binding protein according to any one of claims 56-78, optionally wherein the cell is genetically engineered.

90. The host cell of claim 89, wherein the host cell comprises a chromosomal gene knockout of a TCR gene, an HLA gene, or both.

91. The host cell of claim 89 or 90, wherein the host cell comprises a knockout of an HLA gene selected from an αl macroglobulin gene, α2 macroglobulin gene, α3 macroglobulin gene, β1 microglobulin gene, β2 microglobulin gene, and combinations thereof.

92. The host cell of any one of claims 89-91, wherein the host cell comprises a knockout of a TCR gene selected from a TCR α variable region gene, TCR β variable region gene, TCR constant region gene, and combinations thereof.

93. The host cell of any one of claims 89-92, wherein the host cell expresses CD8α, CD8β, a DN-TGFβRII, and/or a selectable protein marker, optionally wherein the selectable protein marker is DHFR, further optionally wherein the CD8α, CD8β, the DN-TGFβRII, and/or the selectable protein marker is fused to a CD34 enrichment tag.

94. The host cell of claim 93, wherein host cells are enriched using the CD34 enrichment tag.

95. The host cell of any one of claims 89-94, wherein the host cell is a hematopoietic progenitor cell, peripheral blood mononuclear cell (PBMC), cord blood cell, or immune cell.

96. The host cell of claim 95, wherein the immune cell is a T cell, cytotoxic lymphocyte, cytotoxic lymphocyte precursor cell, cytotoxic lymphocyte progenitor cell, cytotoxic lymphocyte stem cell, $CD4^+$ T cell, $CD8^+$ T cell, CD4/CD8 double negative T cell, gamma delta ($\gamma\delta$) T cell, natural killer (NK) cell, NK-T cell, dendritic cell, or a combination thereof.

97. The host cell of any one of claims 89-96, wherein the T cell is a naive T cell, central memory T cell, effector memory T cell, or a combination thereof.

98. The host cell of any one of claims 89-97, wherein the T cell is a primary T cell or a cell of a T cell line.

99. The host cell of any one of claims 89-98, wherein the T cell does not express or has a lower surface expression of an endogenous TCR.

100. The host cell of any one of claims 89-99, wherein the host cell is capable of producing a cytokine or a cytotoxic molecule when contacted with a target cell that comprises a peptide-MHC (pMHC) complex comprising a MAGEC2 peptide epitope in the context of an MHC molecule.

101. The host cell of claim 100, wherein the host cell is contacted with the target cell *in vitro*, *ex vivo*, or *in vivo*.

102. The host cell of claim 100 or 101, wherein the cytokine is TNF-α, IL-2, and/or IFN-γ.

103. The host cell of any one of claims 89-102, wherein the cytotoxic molecule is perforins and/or granzymes, optionally wherein the cytotoxic molecule is granzyme B.

104. The host cell of any one of claims 89-103, wherein the host cell is capable of producing a higher level of cytokine or a cytotoxic molecule when contacted with a target cell with a heterozygous expression of MAGEC2.

105. The host cell of claim 104, wherein the host cell is capable of producing an at least 1.05-fold higher level of cytokine or a cytotoxic molecule.

106. The host cell of any one of claims 89-103 wherein the host cell is capable of killing a target cell that comprises a peptide-MHC (pMHC) complex comprising the MAGEC2 peptide epitope in the context of an MHC molecule.

107. The host cell of claim 106, wherein the killing is determined by a killing assay.

108. The host cell of claim 106 or 107, wherein the ratio of the host cell and the target cell in the killing assay is from 20:1 to 1:4.

109. The host cell of any one of claims 106-108, wherein the target cell is a target cell pulsed with 1 μg/mL to 50 pg/mL of MAGEC2 peptide, optionally wherein the target cell is a cell monoallelic for an MHC matched to the MAGEC2 peptide.

110. The host cell of any one of claims 106-109, wherein the host cell is capable of killing a higher number of target cells when contacted with target cells with a heterozygous expression of MAGEC2, optionally wherein the cell killing is at least 1.05-fold higher.

111. The host cell of any one of claims 89-110, wherein the target cell is cell line or a primary cell, optionally wherein the target cell is selected from the group consisting of a HEK293 derived cell line, a cancer cell line, a primary cancer cell, a transformed cell line, and an immortalized cell line.

112. The host cell of any one of claims 89-111, wherein the MAGEC2 immunogenic peptide is as according to any one of claims 1-4 and/or wherein the MHC or MHC-peptide complex is as according to any one of claims 8-17.

113. The host cell of any one of claims 89-112, wherein the host cell does not induce T cell expansion, cytokine release, or cytotoxic killing when contacted with a target cell that comprises a peptide-MHC (pMHC) complex comprising a CLHC1 and/or SLC26A7 peptide epitope.

114. The host cell of any one of claims 89-113, wherein the host cell does not express MAGEC2 antigen, is not recognized by a binding protein of any one of claims 56-78, is not of serotype HLA-B*07 and/or does not express an HLA-B*07 allele.

115. A population of host cells according to any one of claims 89-114.

116. A composition comprising a) a binding protein according to any one of claims 56-77, b) an isolated nucleic acid according to claim 80 or 81, c) a vector according to any one of

claims 82-88, d) a host cell according to any one of claims 89-114, and/or e) a population of host cells according to claim 115, and a carrier.

117. A device or kit comprising a) a binding protein according to any one of claims 56-77, b) an isolated nucleic acid according to claim 80 or 81, c) a vector according to any one of claims 82-88, d) a host cell according to any one of claims 89-114, and/or e) a population of host cells according to claim 115, said device or kit optionally comprising a reagent to detect binding of a), d) and/or e) to a pMHC complex.

118. A method of producing a binding protein according to any one of claims 56-77, wherein the method comprises the steps of: (i) culturing a transformed host cell which has been transformed by a nucleic acid comprising a sequence encoding a binding protein according to any one of claims 56-77 under conditions suitable to allow expression of said binding protein; and (ii) recovering the expressed binding protein.

119. A method of producing a host cell expressing a binding protein according to any one of claims 56-77, wherein the method comprises the steps of: (i) introducing a nucleic acid comprising a sequence encoding a binding protein according to any one of claims 56-77 into the host cell; and (ii) culturing the transformed host cell under conditions suitable to allow expression of said binding protein.

120. A method of detecting the presence or absence of a MAGEC2 antigen and/or a cell expressing MAGEC2, optionally wherein the cell is a hyperproliferative cell, comprising detecting the presence or absence of said MAGEC2 antigen in a sample by use of at least one binding protein according to any one of claims 56-77, at least one host cell according to any one of claims 89-114, or a population of host cells according to claim 115, wherein detection of the MAGEC2 antigen is indicative of the presence of a MAGEC2 antigen and/or cell expressing MAGEC2.

121. The method of claim 120, wherein the at least one binding protein, or the at least one host cell, forms a complex with the MAGEC2 peptide in the context of an MHC molecule, and the complex is detected in the form of fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

122. The method of claim 120 or 121, further comprising obtaining the sample from a subject.

123. A method of detecting the level of a disorder characterized by MAGEC2 expression in a subject, comprising:

a) contacting a sample obtained from the subject with at least one binding protein according to any one of claims 56-77, at least one host cell according to any one of claims 89-114, or a population of host cells according to claim 115; and

b) detecting the level of reactivity,

wherein the presence or a higher level of reactivity compared to a control level indicates the level of the disorder characterized by MAGEC2 expression in the subject.

124. The method of claim 123, wherein the control level is a reference number.

125. The method of claim 123 or 124, wherein the control level is a level from a subject without the disorder characterized by MAGEC2 expression.

126. A method for monitoring the progression of a disorder characterized by MAGEC2 expression in a subject, the method comprising:

a) detecting in a subject sample the presence or level of reactivity between a sample obtained from the subject and at least one binding protein according to any one of claims 56-77, at least one host cell according to any one of claims 89-114, or a population of host cells according to claim 115;

b) repeating step a) at a subsequent point in time; and

c) comparing the level of MAGEC2 or the cell of interest expressing MAGEC2 detected in steps a) and b) to monitor the progression of the disorder characterized by MAGEC2 expression in the subject, wherein an absent or reduced MAGEC2 level or the cell of interest expressing MAGEC2 detected in step b) compared to step a) indicates an inhibited progression of the disorder characterized by MAGEC2 expression in the subject and a presence or increased MAGEC2 level or the cell of interest expressing MAGEC2 detected in step b) compared to step a) indicates a progression of the disorder characterized by MAGEC2 expression in the subject.

127. The method of claim 126, wherein between the first point in time and the subsequent point in time, the subject has undergone treatment to treat the disorder characterized by MAGEC2 expression.

128. A method for predicting the clinical outcome of a subject afflicted with a disorder characterized by MAGEC2 expression comprising:

a) determining the presence or level of reactivity between a sample obtained from the subject and at least one binding protein according to any one of claims 56-77, at least one host cell according to any one of claims 89-114, or a population of host cells according to claim 115; and

b) comparing the presence or level of reactivity to that from a control, wherein the control is obtained from a subject having a good clinical outcome;

wherein the absence or a reduced level of reactivity in the subject sample as compared to the control indicates that the subject has a good clinical outcome.

129. A method of assessing the efficacy of a therapy for a disorder characterized by MAGEC2 expression comprising:

a) determining the presence or level of reactivity between a sample obtained from the subject and at least one binding protein according to any one of claims 56-77, at least one host cell according to any one of claims 89-114, or a population of host cells according to claim 115, in a first sample obtained from the subject prior to providing at least a portion of the therapy for the disorder characterized by MAGEC2 expression to the subject, and

b) determining the presence or level of reactivity between a sample obtained from the subject and at least one binding protein according to any one of claims 56-77, at least one host cell according to any one of claims 89-114, or a population of host cells according to claim 115, in a second sample obtained from the subject following provision of the therapy for the disorder characterized by MAGEC2 expression,

wherein the absence or a reduced level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is efficacious for treating the disorder characterized by MAGEC2 expression in the subject, and wherein the presence or an increased level of reactivity in the second sample, relative to the first sample, is an indication that the therapy is not efficacious for treating the disorder characterized by MAGEC2 expression in the subject.

130. The method of any one of claims 120-129, wherein the level of reactivity is indicated by a) the presence of binding and/or b) T cell activation and/or effector function, optionally wherein the T cell activation or effector function is T cell proliferation, killing, or cytokine release.

131. The method of any one of claims 120-130, wherein the T cell binding, activation, and/or effector function is detected using fluorescence activated cell sorting (FACS), enzyme linked immunosorbent assay (ELISA), radioimmune assay (RIA), immunochemically, Western blot, or intracellular flow assay.

132. A method of preventing and/or treating a disorder characterized by MAGEC2 expression comprising contacting target cells expressing MAGEC2 with a therapeutically effective amount of a composition comprising cells expressing at least one binding protein according to any one of claims 56-77, optionally wherein the composition is administered to a subject.

133. The method of any one of claims 49-55 and 132, wherein the cell is an allogeneic cell, syngeneic cell, or autologous cell.

134. The method of any one of claims 49-55, 132, and 133, wherein the cell is a host cell according to any one of claims 89-114 or a population of host cells according to claim 115.

135. The method of any one of claims 49-55 and 132-134, wherein the target cell is a cancer cell expressing MAGEC2.

136. The method of any one of claims 49-55 and 132-135, wherein the composition further comprises a pharmaceutically acceptable carrier.

137. The method of any one of claims 49-55 and 132-136, wherein the composition induces an immune response against the target cell expressing MAGEC2 in the subject.

138. The method of any one of claims 49-55 and 132-137, wherein the composition induces an antigen-specific T cell immune response against the target cell expressing MAGEC2 in the subject.

139. The method of any one of claims 49-55 and 132-138, wherein the antigen-specific T cell immune response comprises at least one of a $CD4^+$ helper T lymphocyte (Th) response and a CD8+ cytotoxic T lymphocyte (CTL) response.

140. The method of any one of claims 49-55 and 132-139, further comprising administering at least one additional treatment for the disorder characterized by MAGEC2 expression, optionally wherein the at least one additional treatment for the disorder characterized by MAGEC2 expression is administered concurrently or sequentially with the composition.

141. The method of any one of claims 132-140, wherein the disorder characterized by MAGEC2 expression is a cancer or relapse thereof, optionally wherein the cancer is selected from the group consisting of melanoma, head & neck cancer, lung cancer, cervical cancer, prostate cancer, multiple myeloma, hepatocellular carcinoma, breast invasive carcinoma, and bladder urothelial carcinoma.

142. The method of any one of claims 132-141, wherein the subject is an animal model of a disorder characterized by MAGEC2 expression and/or a mammal, optionally wherein the mammal is a human, a primate, or a rodent.