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(54) Title: MAJOR HISTOCOMPATIBILITY COMPLEX-BASED CHIMERIC RECEPTORS AND USES THEREOF FOR
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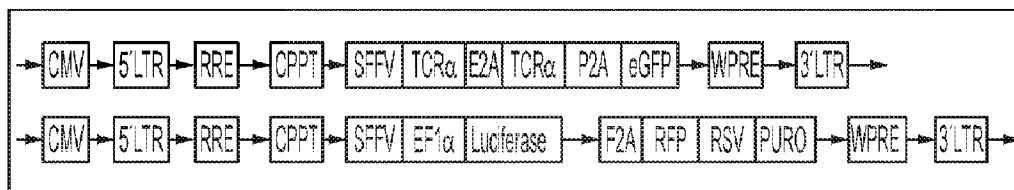


FIG. 1

(57) Abrégé/Abstract:

Major histocompatibility complex-based chimeric receptors (MHC-CAR) for use in targeting autoreactive immune cells. Also provided herewith are genetically engineered immune cells expressing the MHC-CAR for use in treating autoimmune diseases such as multiple sclerosis.

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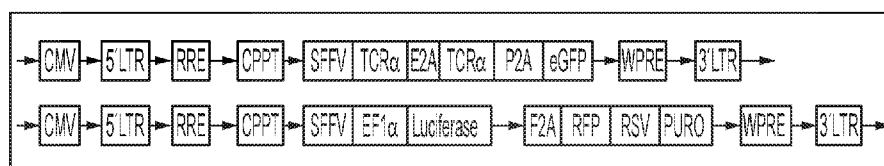


FIG. 1

(57) Abstract: Major histocompatibility complex-based chimeric receptors (MHC-CAR) for use in targeting autoreactive immune cells. Also provided herewith are genetically engineered immune cells expressing the MHC-CAR for use in treating autoimmune diseases such as multiple sclerosis.



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MAJOR HISTOCOMPATIBILITY COMPLEX-BASED CHIMERIC RECEPTORS AND USES THEREOF FOR TREATING AUTOIMMUNE DISEASES

RELATED APPLICATION

This application claims the benefit of the filing date of U.S. Provisional Application Serial No. 62/584,449, filed on November 10, 2017. The entire contents of this referenced application are incorporated by reference herein.

BACKGROUND OF THE INVENTION

Autoimmune diseases are characterized by abnormal immune responses against self-antigens, leading to damage or disruption of tissues. Multiple sclerosis (MS) is a central nervous system autoimmune disease, in which activated autoreactive T cells invade the blood brain barrier, initiating an inflammatory response that leads to myelin destruction and axonal loss. Although the etiology of MS, the mechanisms associated with its onset and progression, and determination of its outcome remains unelucidated, all available evidence suggests that therapies specifically targeting the pathologic immune cells responsible for MS would have improved therapeutic outcomes over available therapies. Reinhard et al., *Proceedings of the National Academy of Sciences*, 101(suppl 2):14599–14606; 2004. This strategy could be extended to other immune disorders with similar mechanisms, including rheumatoid arthritis. Carol et al., *Nature Reviews Immunology*, 2(2):85–95, 2002.

The major histocompatibility complex (MHC), known as human leukocytes (HLA) in humans, is a set of cell surface proteins essential for the immune system to recognize foreign agents. MHC complexes bind to antigens derived from pathogens and display such to T cells, which are then activated, leading to elimination of cells displaying foreign antigens. MHC complexes may also display intact, and in some cases misfolded, host-derived proteins to B cells thereby inducing the autoantibody responses characteristic of autoimmune disorders. Jiang et al., *International immunology*, 25(4):235-246 (2013), and Busch et al., *The EMBO journal*, 15(2):418, (1996).

SUMMARY OF THE INVENTION

In aspect, the disclosure features a major histocompatibility complex (MHC)-based chimeric receptor (CAR), comprising: (i) an extracellular domain of a MHC molecule conjugated to an antigenic peptide from an antigen involved in an autoimmune disease; and (ii) a cytoplasmic signaling domain, at least one co-stimulatory domain, or a combination

thereof. The MHC-based CAR may further comprises a hinge domain located between (i) and (ii). The antigenic peptide is dependent on the autoimmune disorder and may be from myelin basic protein (MBP), proteolipid protein (PLP), insulin, glutamate decarboxylase, or the additional exemplary self-antigens as described in Table 1.

In some examples, the MHC-based chimeric receptor comprises at least one co-stimulatory domain, which may be a co-stimulatory domain from 4-1BB (CD137), a co-stimulatory domain from CD28, or a combination thereof. In other examples, the MHC-based chimeric receptor as described herein may be free of a cytoplasmic signaling domain. Alternatively or in addition, the MHC-CAR comprises a cytoplasmic signaling domain of CD3 ζ .

In some embodiments, the MHC molecule in the MHC-CAR is a class I MHC, for example, a human class I MHC. In some instances, the extracellular domain of the chimeric receptor comprises an extracellular domain of the alpha chain of the class I MHC, which is fused to the antigenic peptide. For example, the chimeric receptor may be a fusion polypeptide comprising (i) the extracellular domain of the class I MHC molecule, and (ii) the cytoplasmic domain, the at least one co-stimulatory domain, or the combination thereof. In one example, the chimeric receptor is a fusion polypeptide, which comprises, from N-terminus to C-terminus, a signal peptide, a first peptide linker, the antigenic peptide, a second peptide linker, an extracellular domain of macroglobulin, a third peptide linker, the class I MHC molecule, a transmembrane domain, the at least one co-stimulatory domain, and CD3 ζ .

In other embodiments, the MHC-based chimeric receptor as described herein comprises a class II MHC (*e.g.*, a human MHC II) or a portion thereof. Such a chimeric receptor may comprise a first polypeptide, which comprises an extracellular domain of a first MHC class II, and a second polypeptide, which comprises an extracellular domain of a beta chain of a second MHC class II, and wherein the antigenic peptide is fused to either the first polypeptide or the second polypeptide, and wherein either the first polypeptide or the second polypeptide further comprises the cytoplasmic signaling domain, the at least one co-stimulatory domain, or the combination thereof. In some examples, the chimeric receptor can be a fusion polypeptide comprising (i) an extracellular domain of the alpha chain of a first MHC class II molecule, (ii) an extracellular domain of the beta chain of a second MHC class II molecule, (iii) the antigenic peptide, and (iv) the cytoplasmic signaling domain, the at least one co-stimulatory domain, or the combination thereof. In some examples the antigenic protein may not be linked to the MHC class II and may instead be expressed as a separate

fusion polypeptide with an alternative signal peptide (such as that from CD150, *i.e.*, MDPKGLLSLTFVLFLSLAFG (SEQ ID NO: 388)). In some examples, the first MHC class II is HLA-DRA*1010. Alternatively or in addition, the second MHC class II is HLA-DRB1*1501.

In another aspect, the present disclosure features a nucleic acid or a nucleic acid set, which collectively encodes any of the MHC-based chimeric receptors described herein. In some instances, the nucleic acid or nucleic acid set can be located in one or more vectors, for example, viral vector(s).

Further, the present disclosure provides a genetically modified immune cell (*e.g.*, a T cell), which expresses any of the MHC-based chimeric receptors described herein. In some instances, the activity of the endogenous T cell receptor (TCR) can be suppressed, which may be achieved by mutating or deleting the alpha chain of the endogenous TCR, the beta chain of the endogenous TCR, or both to disrupt surface expression of the endogenous TCR. Alternatively or in addition, the expression of the endogenous CD52 can be disrupted.

In some embodiments, the genetically modified immune cell as described herein may further express a suicide gene (*e.g.*, RQR8), a marker gene (*e.g.*, GFP), or both. When necessary, the immune cell can be further modified for lymph node or tertiary lymphoid organ delivery and retention. For example, the immune cell can be further engineered to overexpress VAP-1, L-selectin, CCR7, CXCR5, or a combination thereof. In some instances, the expression of endogenous sphingosine-1-phosphate receptor 1 can be disrupted in the genetically modified immune cell.

In some embodiments, the immune cell can be engineered to travel to the site of inflammation, for instance using a chemokine receptor such as CCR6 (*e.g.*, to the site of Th17 cells), CXCR3 or CXCR4 (*e.g.*, to the site of plasma cells), or through a membrane linked, antigen targeted antibody. Alternatively or in addition, the genetically modified immune cell may further comprise a genetic modification that results in blockade of PD-1 signaling. If needed and the disorder is especially severe the MHC-CAR cells can also be designed to remove or inactive bystander B cells (with a CD19 or CD20-CAR) or plasma cells (with a CS1-CAR and/or CS1 knockout).

In some embodiments, the genetically modified immune cell as described herein may be a regulatory T cell, which can be CD25+, and optionally CD4+. In some instances, the regulatory T cell can be derived from CD25++CD45R+ T cells isolated from peripheral blood mononuclear cells or from cord blood. In other instance, the regulatory T cell may

comprise a transgene coding for CD25. Any of the Treg cells disclosed herein may further express a chimeric receptor specific to CD19, a chimeric receptor specific to CS-1, or both. Alternatively or in addition, the regulatory T cell may further express CCR6, CXCR5, PD-1, or a combination thereof. In some examples, the regulatory T cell may display an antibody specific to MOG.

In yet another aspect, the present disclosure provides a method for suppressing autoreactive immune cells in a subject having an autoimmune disease (*e.g.*, multiple sclerosis). The method may comprise administering to the subject an effective amount of genetically modified immune cells as described herein, which can be T cells.

In some embodiments, the genetically modified immune cells are autologous. In other embodiments, the genetically modified immune cells are allogeneic. Any of the genetically modified immune cells may be administered to a lymph node of the subject. In some instances, the subject is undergoing a therapy comprising an antibody specific to CD52.

In some embodiments, the subject is a human patient having or at risk for multiple sclerosis and the genetically modified T cells are Treg cells or cytotoxic lymphocytes (CTLs) as described herein.

In some examples, the human patient is an early-stage MS patient and the Treg cells express the MHC-CAR and have one or more of the following genetic modifications: (i) PD-L1 and/or PD-1 knockout; (ii) surface expression of CCR6 and/or CXCR5; (iii) surface display of an antibody or an antigen-binding fragment thereof that is specific to MOG; and (iv) surface expression of a chimeric receptor targeting CD19. In some examples the patient may first, simultaneously, or alternatively be treated with cytotoxic CTLs with modifications of the same type.

In some examples, the human patient has relapsing-remitting MS or early-stage progressive MS and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface display of an antibody or antigen binding fragment that is specific to MOG; and (ii) surface expression of CCR6. In some examples the patient may first, simultaneously, or alternatively be treated with cytotoxic CTLs with modifications of the same type.

In some examples, the human patient has relapsing-remitting MS or early-stage progressive MS and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CD19; and (ii) surface expression of CXCR5. In some examples the patient may first, simultaneously, or

alternatively be treated with CTLs with the same modifications.

In some examples, the human patient has MS in chronic progressive form and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CS-1; and (ii) surface expression of an agent CXCR4, CCR6, and/or CXCR5. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

In some embodiments, the subject is a human patient having or at risk for systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis (also known as juvenile idiopathic arthritis), Sjögren's syndrome, systemic sclerosis, ankylosing spondylitis, Type 1 diabetes, autoimmune thyroid diseases (Grave's and Hashimoto's), multiple sclerosis myasthenia gravis, inflammatory bowel disease (Crohn's or ulcerative colitis), Psoriasis, or a diseases mentioned in Table 1 and the genetically modified T cells are Treg cells and/or CTLs as described herein.

In some examples, the human patient is an early-stage patient of any of the autoimmune disorders described herein (*e.g.*, those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following genetic modifications: (i) PD-L1 and/or PD-1 knockout; (ii) surface expression of CCR6 and/or CXCR5; (iii) surface display of an antibody or an antigen-binding fragment thereof that is specific to a relevant protein described as an autoantigen in Table 1 for that autoimmune disorder; and (iv) surface expression of a chimeric receptor targeting CD19. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

In some examples, the human patient has moderately severe disease state of any of the autoimmune disorders as described herein (*e.g.*, those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface display of an antibody or antigen binding fragment that is specific to a relevant protein described as an autoantigen in Table 1 for that autoimmune disorder; and (ii) surface expression of CCR6. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

In some examples, the human patient has moderately severe disease state of any of the autoimmune disorders as described herein (*e.g.*, those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CD19; and (ii) surface expression of CXCR5. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs

with the same modifications.

In some examples, the human patient has severe disease state of any of the autoimmune disorders described herein (*e.g.*, those listed in Table 1) and the Treg cells express the MHC-CAR and have one or more of the following modifications: (i) surface expression of a chimeric receptor targeting CS-1; and (ii) surface expression of an agent targeting CXCR4, CCR6, and/or CXCR5. In some examples the patient may first, simultaneously, or alternatively be treated with CTLs with the same modifications.

Also within the scope of the present disclosure are pharmaceutical compositions for use in treating an autoimmune disease, the composition comprising genetically modified immune cells expression MHC-CAR as described herein such as Treg cells and a pharmaceutically acceptable carrier, and uses of such genetically modified immune cells for manufacturing a medicament for use in treating the target autoimmune disease.

The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of an exemplary design of a lentiviral expression vector for expression of an antigen-specific T cell receptor (TCR). mRNA and multicistronic mRNA designs are similar.

Figure 2 is a schematic illustration of an MHC-CAR T cell, which expresses various designs of MHC-CAR as indicated in conjugation with a myelin basic protein (MBP) peptide for targeting pathologic T cells involved in multiple sclerosis (MS). Optionally, the MHC-CAR T cell may have the endogenous TCR and/or CD52 knocked out. The MHC-CAR T cell may further express RQR8 on the cell surface.

Figure 3 is a schematic illustration of exemplary designs for various MHC-CAR constructs. The exemplary MHC-CAR constructs may have two subunits: an α -chain containing a leader sequence, a DRA*1010 domain, and a cytoplasmic domain, and a β -chain that includes a leader sequence from HLA-DRB1*1501, a peptide from MBP, and a domain from HLA-DRB1*1501. The DNA used to create mRNA contains either single chains or are multicistronic and separated by orthogonal 2A sequences. RQR8 and eBFP (or GFP) are used for both cell control and labeling.

Figure 4 is a schematic illustration of exemplary designs of expression cassettes for various MHC-CAR constructs containing a MBP peptides, which may further include eBFP (or GFP) or RQR8.

Figure 5 is a schematic illustration of an exemplary design of a lentiviral expression construct for MHC-CARs and optionally label proteins such as eBFP and/or RQR8. Such an expression cassette would be sufficiently small to be included in one lentiviral package.

Figure 6 is a schematic illustration of an exemplary design of MHC-CAR, which may include a number of sites for tagging. Site 1 is an HLA-DR antibody binding site for cases where naïve HLA-DR is either not expressed or due to CIITA editing. Sites 2 and 3 are potential insertion sites for polyhistidine-tag motifs. Sites 4 and 5 represent RQR and RQR8, respectively.

Figure 7 is a schematic illustration of exemplary designs of MHC Class II moieties linked to antigenic peptides. “N” refers to the N-terminus of a polypeptide. Circled black dots refer to the antigenic peptides.

Figure 8A and Figure 8B are schematic illustrations of MHC Class I exemplary constructs. Figure 8A depicts exemplary designs of MHC Class I moieties linked to antigenic peptides. “N” refers to the N-terminus of a polypeptide. Circled black dots refer to the antigenic peptides. Figure 8B depicts an exemplary expression cassette for a MHC Class I CAR construct.

Figure 9 depicts exemplary co-stimulatory domains and combinations thereof for constructing the MHC-CAR and considerations for co-expression of cytokines.

Figure 10 depicts exemplary single-chain and multi-chain MHC Class I and Class II MHC-CAR constructs, including multi-chain MHC-CAR constructs containing both MHC Class I and Class II components.

Figure 11 is a plot showing CD3 expression on primary human stimulated CD3+ T cells (TCELL-0028) transfected with an mRNA encoding Cas9 (“Cas9 only”) or an mRNA encoding Cas9 (“Cas9 only”) and sgRNAs targeting the gene encoding T cell receptor alpha domain (TRAC) (“Cas9 + TRAC gRNA”).

Figure 12 is a plot showing relative cell viability in a killing assay. HEK-293 cells transfected with Construct 1, Construct 2, or both Constructs 1 and 2 were incubated with media only, rituximab, complement, or both rituximab and complement.

DETAILED DESCRIPTION OF THE INVENTION

Autoreactive T cells, (*e.g.*, those for myelin components involved in multiple sclerosis) exist in normal individuals. The majority of determinant of disease induction is in the class of immune response that occurs when these autoreactive T cells are triggered in autoimmune patients (*e.g.*, in MS patients). Generation of pathologic autoreactive T cells is favored both by specific major histocompatibility complex (MHC) and non-MHC genes, which determine the protein sequences an individual reacts against and the class of the immune response.

Once an immune attack begins on an initial autoantigen (for example, a myelin antigen in MS), there is a spreading of reactivity to other autoantigens; that is, if a T cell attacks one autoantigen (for example, a brain protein in MS), other structures are damaged and they can sensitize additional T cells to attack other targets in a process called “epitope-spreading”, a process that is shared by all autoimmune disorders and common to disease response in general.

B cells are ordinary components of the immune reaction in the early disease lesion caused by initial autoreactive attacks, for example, active MS lesion as well. B cell accumulation occurs as packed aggregates or ectopic B cell follicles. Serafini et al., *Brain Pathol.* 14: 164–144 (2004); Wekerle, *Autoimmunity*, 50:1, 57-60 (2017); and Pröbstel, et al., *International journal of molecular sciences*, 16(7), pp.16576-16592 (2015). In MS, B cells were reported to be found in the brain and spinal cord of RR-, SP-, and P-stage MS patients. Therapeutic treatments that target B cells either directly or indirectly have proven beneficial in treatment of autoimmune diseases such as MS. Wekerle, 2017.

Both CD4+ and CD8+ T cells are present in MS lesions and are believed to play a central role in disease development. Increased frequencies of myelin-reactive (MBP, PLP, and MOG) CD4 and CD8 cells are found in MS patients compared to healthy controls. Cao, et al., *Sci. Transl. Med.* 7 (287), 287ra74 (2014); Martin, et al., *J. Exp. Med.* 173 (Jan 1, 1991); Ota, et al., *Nature* 346, 183 (Jul 12, 1990); Pette, et al., *Neurology* 40, 1770 (1990); and Raddassi, et al., *J. Immunol.* 187, 1039 (2011).

Th1 cells producing IFN-gamma and Th17 cells are uniquely pathogenic. Factors that favor the development of Th1 cells are elevated in MS patients and are also triggered by viral infections: gamma interferon; IL-12 – in almost all treatments that affect the immune system and help MS, almost all decrease Th1 response and increase Th2 and TH3 response. Th17 cells are present at sites of tissue inflammation and are implicated in

autoimmune/chronic inflammatory conditions. Th17 producing CD4 and CD8 cells are increased in the lesions, blood, and CSF of patients. [Tzartos 2008; Matusevicius 1999; Bruchlacher-Waldert 2009]. The CCR6 and CD161 on Th17 cells are hypothesized to be homing molecules to inflamed tissues [Cosmi, 2008].

Th17 cells are also implicated in a number of other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis (also known as juvenile idiopathic arthritis), Sjögren's syndrome, systemic sclerosis, ankylosing spondylitis, Type 1 diabetes, autoimmune thyroid diseases (Grave's and Hashimoto's), myasthenia gravis, inflammatory bowel disease (Crohn's or ulcerative colitis), and psoriasis. Tabarkiewicz et al., *Archivum immunologiae et therapiae experimentalis*, 63(6):435-449 (2015).

The ultimate goal of any treatment for autoimmune disease is a complete suppression of pathology. In the case of multiple sclerosis and other autoimmune disorders, pathologic lymphocytes (both B and T cells, and if necessary plasma cells for very severe cases) are expected to be eliminated or controlled to halt the disease course, and interventions at different stages of disease progression require different cellular targets and therefore therapeutic cells.

Disclosed herein are major histocompatibility complex (MHC)-based chimeric receptors (MHC-CAR) for targeting autoreactive immune cells such as autoreactive T cells. A MHC-CAR as described herein comprises one or more MHC polypeptides or an extracellular domain thereof and one or more cell signaling domains, for example, a cytoplasmic signaling domain (*e.g.*, that from CD3 ζ), at least one co-stimulatory domain (*e.g.*, that from 4-1BB or CD28), or both. The MHC-CAR may further comprise an antigenic peptide from an autoantigen or a foreign antigen that mimics an autoantigen in eliciting autoimmune responses. Also herein are nucleic acids encoding the MHC-CAR, vectors carrying such, and genetically engineered immune cells such as T cell and natural killer (NK) cells expressing the MHC-CAR. Such genetically engineered immune cells can be used to target autoreactive immune cells, thereby benefiting treatment of autoimmune diseases involving the autoreactive immune cells.

Also disclosed herein are genetically modified regulatory T (Treg) cells expressing an MHC-based chimeric receptor as disclosed herein. Such Treg cells may be further modified with chimeric receptor(s) targeting T cell and/or B cell surface markers, as well as additional genetic engineering for, *e.g.*, targeting specific tissue sites (*e.g.*, lymph nodes or inflammation

sites) or modulating immune responses (*e.g.*, checkpoint modulation). The genetically modified Treg cells may be used to inhibit pathogenicity at an early stage of a target disease, to control disease progression at a middle stage of the disease (*e.g.*, relapsing or remitting MS), or to suppress pathology via, *e.g.*, inducing cytotoxicity of pathologic CD8+ T cells at a late stage of the disease (*e.g.*, chronic progressive MS).

I. Major histocompatibility complex (MHC)-based chimeric receptors (MHC-CARs)

The MHC based chimeric receptor (MHC-CAR) described herein comprises an MHC moiety, which is conjugated to an antigenic peptide (*e.g.*, a misfolded one), and at least one cell signaling moiety, which can be a cytoplasmic signaling domain (*e.g.*, that of CD3 ζ), one or more co-stimulatory domains (*e.g.*, that of 4-1BB or CD28), or a combination thereof. In some instances, the antigenic peptide can be part of a fusion polypeptide of the MHC-CAR. In other instances, the antigenic peptide does not form a fusion polypeptide with the MHC-CAR but forms a complex with the MHC-CAR. As used herein, the term “conjugated” means that at least two components are physically associated, either *via* covalent bonds or *via* non-covalent interactions.

In some examples, the MHC-CAR can be a single fusion polypeptide containing the MHC moiety, the antigenic peptide, and the at least one cell signaling moiety. Such a single fusion polypeptide may form complexes with endogenous cell membrane proteins (*e.g.*, β -microglobulin) when expressed in a suitable immune cells.

In other examples, the MHC-CAR described herein may be a multi-chain protein complex, for example, a heterodimer, comprising one polypeptide that comprises the antigenic peptide. In some instances, the antigenic peptide or polypeptide may be expressed as a separate polypeptide, which may form a complex (*e.g.*, a trimer) with the MHC components. The antigenic polypeptide can be a misfolded antigenic protein that binds to the MHC. Optionally, the MHC-CAR may further comprise a hinge domain, which may be adjacent to the antigenic peptide and/or the MHC moiety, a signal peptide at the N-terminus, and/or one or more tagging sites, for example, a histidine protein tag and/or an RQR domain that additionally acts as a kill-switch site.

(i) Components of MHC-CARs

(a) MHC Moiety

The MHC-CAR constructs disclosed herein comprise an MHC moiety, which may comprise one or more MHC polypeptides or an extracellular domain thereof. The MHC

moiety may be derived from a suitable source, for example, human or a non-human mammal (*e.g.*, monkey, mouse, rat, rabbit, pig, etc.) In some instances, the MHC moiety is from a human MHC molecule (also known as HLA). In some instances the domains that interact with molecules from other cells (TCR or BCR) are from a human MHC molecule. There are primarily two classes of MHC molecules, MHC class I molecules and MHC class II molecules, both of which can be used for constructing the MHC-CARs described herein. Sequences of MHC class I and class II molecules of various species (*e.g.*, human, non-human primates, canids, fish, ovids, bovines, equids, suids, murids, and gallus) are available from public gene datasets, for example, the IPD-MHC database and the IMGT/HLA database provided by EMBL-EBI and the dbMHC database provided by National Center for Biotechnology Information (NCBI).

MHC class I molecules are heterodimers containing an alpha chain and β -microglobulin. The extracellular domain of an alpha chain includes three subdomains, $\alpha 1$, $\alpha 2$, and $\alpha 3$. In some embodiments, the MHC moiety may include the alpha chain of a MHC class I molecule, or an extracellular domain thereof, for example, the $\alpha 1$ domain, the $\alpha 2$ domain, the $\alpha 3$ domain, or a combination thereof. The MHC class I molecule may be a human HLA-A molecule, a human HLA-B molecule, or a human HLA-C molecule. In some instances, the alpha chain of the MHC class I molecule may be fused with β -microglobulin to produce a single chain fusion protein. In some examples, the MHC Class I moiety is from HLA A3, which can be co-used with a PLP peptide. Honma et al., *J. Neuroimmunol.* 73:7-14 (1997). In other examples, the MHC Class I is from HLA A2, which can be used with the same PLP peptide and display of a viral peptide such as TAX. TAX is from the protein tax or p40 (Genbank accession no. BAB20130.1) that is a molecular mimic of a human neuronal protein and from the HTLV-1 virus, which is implicated in diseases such as rheumatoid arthritis, system lupus erythematosus, and Sjogren's syndrome. Garboczi, et al. *The Journal of Immunology*, 157(12):5403-5410, 1996. Quaresma, et al., 2015. *Viruses*, 8(1):5 2015. The class I protein and peptide may additionally contain modifications to enable more robust peptide loading such replacement of the invariant tyrosine at position 84 of the heavy chain with alanine; or alternatively the position 84 tyrosine can be replaced with cysteine as can the second position of the peptide- $\beta 2m$ linker to create a disulfide trap. Hansen et al. *Trends in immunology*, 31(10):363 (2010).

Like MHC class I molecules, MHC class II molecules are also heterodimers consisting of two homogenous peptides, an α -chain and a β -chain. The extracellular domain

of each of the α -chain and the β -chain contains two subdomains $\alpha 1/\alpha 2$, and $\beta 1/\beta 2$. When a MHC class II molecule is used for constructing a MHC-CAR, the MHC moiety may include two subunits, one including the α -chain or a portion thereof, for example, an extracellular domain thereof (*e.g.*, $\alpha 1$, $\alpha 2$, or both), the other including the β -chain or a portion thereof, for example, an extracellular domain thereof (*e.g.*, $\beta 1$, $\beta 2$, or both). In cases where only the region that interacts with other cell types is used (*i.e.*, $\alpha 1$ and $\beta 1$), specific amino acid modifications may be required to enhance the folding of the mini-MHC, see mini-sequence with shaded regions and Birnbaum et al. The MHC class II molecule may be a human HLA DP molecule, a human HLA DM molecule, a human HLA DOA molecule, a human HLA DOB molecule, a human HLA DQ molecule, or a human HLA DR molecule. In some examples, the MHC class II molecule is a human HLA DR molecule, for example HLA DR*1501.

(b) Antigenic Peptides

The antigenic peptides of the MHC-CAR described herein are an antigenic peptide that is recognizable by pathogenic immune cells (*e.g.*, autoreactive T cells or B cells) involved in an autoimmune disease. When presented by a suitable MHC molecule, such an antigenic peptide would interact with the antigen-specific T cell receptors of pathogenic T cells, leading to downstream immune responses.

In some instances, a specific antigenic peptide can be designed for a specific autoimmune disease patient such as an MS patient, using methods known in the art. Programs like NetMHC enable personalized design of antigenic peptides that are specific to the patient's MHC, and have been used to develop personalized cancer vaccines. Hacohen et al., *Cancer immunology research*, 1(1):11-15 (2013). Also within the scope of the present disclosure are personalized CAR T and Treg therapies for autoimmune disorders. For disorders with very strong MHC associations (such as MS), a personalized therapy can be utilized to treat a large patient class at different stages of the disease. Recent studies have also demonstrated that Class II MHCs and specifically the HLAs implicated in autoimmune disorders can display entire antigenic proteins rather than just processed peptides. Jiang et al., *International immunology*, 25(4):235-246, (2013). These MHC-protein complexes appear to induce autoantibody production in autoimmune disorders, including antibodies that do not bind to properly folded proteins as well as autoantibodies that are specific to those with specific autoimmune disorders. The inventors impute that display of antigenic proteins in MHC-CAR can provide a specific route to remove or deactivate autoimmune specific B

cells, such as those in MS which produce oligoclonal bands whose specificity to proteins has not been unraveled, despite many rigorous attempts. Owens et al., *Annals of neurology*, 65(60):639-649, 2009; Chastre et al., *New England Journal of Medicine*, 374(15):1495-1496, 2016; Housley et al., *Clinical Immunology*, 161(1):51-58, 2015; Larman et al., 2013. *Journal of autoimmunity*, 43:1-9, 2013. In the event that the antigenic protein does not bind the MHC, then that specific MHC-CAR will not be expressed, but as a Treg or CTL it can still play a bystander role in modifying the immune response depending upon its other characteristics and as part of a patient specific population of MHC-CAR T and Treg cells with different specificities.

The antigenic peptides used herein may be fragments of autoantigens involved in autoimmune diseases, for example, myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) involved in multiple sclerosis, insulin and glutamate decarboxylase (GAD) involved in type I diabetes, tryptase involved in rheumatoid arthritis (RA), and the proteins included in Table I below. Alternatively, the antigenic peptide can be a fragment of a pathogen protein such as a viral or a bacterial protein that is highly homologous to a self-antigen involved in an autoimmune disease. Such an antigenic peptide also can target pathogenic T cells. If needed, the antigenic peptide can be a (typically misfolded) antigenic protein or protein fragment that can be expressed separately and binds directly to the MHC moiety of a MHC-CAR described herein. In their natural state (attached to an MHC rather than an MHC moiety of a MHC-CAR), such antigenic protein/MHC complexes stimulate pathogenic B cells to produce autoantibodies. For proteins such as IgGH or rheumatoid factor in rheumatoid arthritis (Jin et al., *Proceedings of the National Academy of Sciences*, 111(10):3787-3792, 2014), β 2-glycoprotein I in antiphospholipid syndrome (Tanimura et al., *Blood*, 125(18):2835-2844, 2015) and recurrent miscarriage (Tanimura et al., *Placenta*, 46:108, 2016), GM-CSF in autoimmune pulmonary alveolar proteinosis (Hamano et al., *ALVEOLAR MACROPHAGE BIOLOGY B32*:A3147-A3147, 2016), tyrosinase in vitiligo (Arase et al. *Journal of Dermatological Science*, 84(1):e87, 2016), and myeloperoxidase in microscopic polyangiitis (Hiwa et al., *Arthritis & Rheumatology*, 69(10):2069-2080, 2017), HLA mediated surface display and in some cases autoantibody binding of misfolded variant/HLA complex can occur.

The antigenic peptides for use in the MHC-CAR described herein may contain up to 20 amino acid residues, the extracellular domain of the antigenic protein, or the full length antigenic protein. When co-used with a MHC class I moiety, the antigenic peptide may be 8-

10 amino acid-long. Such antigenic peptides would fit well into the peptide binding site of a MHC class I molecule. Antigenic peptides to be co-used with MHC class II moieties can be longer, for example, containing 15-24 amino acid residues or up to the full length of the antigenic protein, since the antigen-binding groove of MHC class II molecules is open at both ends, while the corresponding antigen-binding groove on class I molecules is usually closed at each end. The open antigen-binding groove of MHC class II molecules implicated in autoimmune disorders can also frequently display intact (*e.g.*, yet misfolded) antigenic proteins or splice variants. Jiang et al., *International immunology*, 25(4):235-246, 2013.

In some examples, a fragment of human MBP is used for constructing the MHC-CARs described herein. An exemplary amino acid sequence of a human MBP is provided below:

MASQKRPSQRHGSKYLATASTMDHARHGFLPRHRDTGILDSIGRFFGGDRGAPKRGSGKVPWLKPEGRSPL
PSHARSQPGLCNMYKDSHHPARTAHYGS L P Q K S H G R T Q D E N P V V H F F K N I V T P R T P P S Q G K G R G L S L S R
FSWGAEGQRPFGYGGGRASDYKSAHKGFGVDAQGTLSKIFKLGGDRSRSGSPMARRHHHHHH (SEQ ID NO:
1)

Exemplary MBP antigenic peptides include, but are not limited to:

GSKYLATASTMDHARHGFLPRHRDTGILDSIGRFFGGDRG (SEQ ID NO: 2),
KYLATASTMDHARHGFLPRH (SEQ ID NO: 3), ATASTMDHARHGFLPRHRDTGIL (SEQ ID NO: 4),
RDTGILDSIGRFFGGDRGAP (SEQ ID NO: 5), IGRFFGGDRGAPKRGSGKDSHHPARTAHY (SEQ ID
NO: 6), APKRGSGKDSHHAARTAHY (SEQ ID NO: 7), GSGKDSHHPARTAHYGS L P Q (SEQ ID NO:
8), HHPARTAHYGS L P Q K S H G R (SEQ ID NO: 9), HAARTAHYGS L P Q K S Q G H R (SEQ ID NO: 10),
S L P Q K S H G R T Q D E N P V V H F (SEQ ID NO: 11), P Q D E N P V V H F F K N I V T P R T P (SEQ ID NO: 12),
T Q D E N P V V H F F K N I V T P R T P (SEQ ID NO: 13), Q D E N P V V H F F K N I V T P R T P (SEQ ID NO: 14),
D E N P V V H F F K N I V T P R T P P (SEQ ID NO: 15), E N P V V H F F K N I V T P R (SEQ ID NO: 16),
E N P V V H F F K N I V T P R T P (SEQ ID NO: 17), E N P V V H F F K N I V T P (SEQ ID NO: 18),
N P V V H F F K N I V T P R T P P S Q (SEQ ID NO: 19), V V H F F K N I V T P R T (SEQ ID NO: 20),
V V H F F K N I V T P R T P P S Q G K (SEQ ID NO: 21), K N I V T P R T P P S Q G K G R G L (SEQ ID NO: 22),
P S Q G K G R G L S L S R F S W G A E (SEQ ID NO: 23), G K G R G L S L S R F S W G A E G Q R P (SEQ ID NO: 24),
L S R F S W G A E G Q R P F G Y G G (SEQ ID NO: 25), Q R P F G Y G G R A S D Y K S A H K (SEQ ID NO: 26),
A S D Y K S A H K G F K G V D A Q G T (SEQ ID NO: 27), F K G V D A Q G T L S K I F K L G G R (SEQ ID NO: 28),
V D A Q G T L S K I F K L G G R D S R S (SEQ ID NO: 29), and S K I F K L G G R D S R S G S P M A R R (SEQ ID NO:
30).

An example nucleic acid sequence encoding the MBP antigenic peptide of SEQ ID NO: 15 is provided below:

GATGAGAATCCCGTGGTTCATTTTTTTAAGAACATCGTCACACCGCGCACCCACCTG (SEQ ID NO: 411)

Specific examples include MBP13–32, MBP89–101, MBP83–99, MBP111–129, or MBP146–170.

Exemplary amino acid sequences for human myelin oligodendrocyte glycoprotein, proteolipid protein, and myelin associated glycoprotein are provided below:

>CAA52617.1 myelin oligodendrocyte glycoprotein [Homo sapiens]

MASLSRPSLPSCCLCSFLLLLLLQVSSSYAGQFRVIGPRHPFIRALVGDEVELPCRISPGKNATGMEVGWYRPPFSR
VVHLYRNGRDQDGDQAPFYRGRTTELLKDAIGEGKVTLRINVRFSDEGGFTCFRRDHSYQEEAAMELKVEDPFYW
VSPGVLVLLAVLPVLLLQITVGLVFLCLQYRLRGKLRAEIENLHRTFDPHFLRVPCWKITLFVIVPVLGPLVALI
ICYNWLHRRLAGQFLEELRNPF (SEQ ID NO: 31)

Exemplary MOG antigenic peptides include MOG1–20 or MOG35–55.

>AAA60117.1 proteolipid protein [Homo sapiens]

MGLLECCARCLVGAFFASLVATGLCFFGVAFPCGCGHEALTGTE**KLIETYFS**KNYQDYEYLINVIHAFQYVIYGT
ASFFFLYGALLLAEGFYTTGAVRQIFGDYKTTICGKGLSATVTGGQKGRGSRGQHQAHSLELVCTCLGKWLGHDP
KEVGITYALTIVVLLVFACSAVPVYIYFNTWTTCQSIAFPSTKSASIGSLCADARMYGVLPWNAFPGKVCNSLL
SICKTAEFQMTFHLFIAAFVGAATLVSLLTFFMIAATYNEAVLKLMMGRGTFK (SEQ ID NO: 32)

An exemplary antigenic fragment of PLP is underlined and in boldface. Other examples include PLP139–151(4) or PLP178–191.

>AAH93045.1 Myelin associated glycoprotein [Homo sapiens]

MIFLTALPLFWIMI SASRGGHWGAWMPSSI SAFEGTCVSI PCREDFPDEL RPAVVHGVWYFNSPYPKNYPPVVEK
SRTQVVHESFQGRSRLGLDLGRNCTLLLSNVSP ELGGKYYFRGDLGGYNQYTFSEHSVLDIVNTPNIVVPEVV
AGTEVEVSCMVPDNCPELRPELSWLGHGELGEP AVLGRRLREDEGTWVQVSL LHFPVPTREANGHRLGCQASFNTT
LQFEGYASMDVKYPPVIVEMNSSVEAIEGSHVSL LCGADSNPPPLLTWMRDGTVLREAVAESL LLELEEVTPAED
GVYACLAENAYGQDNRTVGLSVMYAPWKPTVNGTMVAVEGETVSILCSTQSNPDPIITIFKEKQILSTVIYSESL
QLELPAVSPEDDGEYWCVAENQYQQRATAFNLSVEFAPV LLLLESHCAAARDTVQCLCVVKSNPESVAFELPSRN
VTVNESEREFEVYSESRGLVLTSLITLRGQAQAPPRVICTARNLYGAKSLELPFQGAHRLMWAKIGPVGAVVAFAL
LIAIVCYITQTTRKKNVTESPSFSAGDNPPVLFSSDFRISGAPEKYESKEVSTLESH (SEQ ID NO: 33)

Table 1 below provides additional exemplary autoantigens associated with other autoimmune diseases.

Table 1. Autoantigens of Various Autoimmune Disorders

Autoantigen	GenBank Accession No.	Associated Autoimmune Disease
Dopachrome tautomerase	AAH28311.1	Alopecia areata
Melanoma antigen gp100	AAC60634.1	Alopecia areata
Melanocyte protein Pmel	NP_001186983.1	Alopecia areata
Melanocyte-stimulating hormone receptor	NP_002377.4	Alopecia areata
Trichohyalin	AAA65582.1	Alopecia areata
Tyrosine 3-monooxygenase	NP_954986.2	Alopecia areata
Amyloid beta A4 protein	NP_000475.1	Alzheimer's
Vasoactive intestinal polypeptide receptor 1	NP_004615.2	Ankylosing spondylitis
Latent membrane protein 2	CAA57360.1	Ankylosing spondylitis
Nitrogenase iron protein	ART03999.1	Ankylosing spondylitis
Aggrecan core protein	NP_001126.3	Ankylosing spondylitis
Beta-2-glycoprotein 1	NP_000033.2	Antiphospholipid syndrome
M protein precursor	AAA26918.1	Antiphospholipid syndrome
Large tegument protein	ACL51127.1	Antiphospholipid syndrome
Steroid 21-hydroxylase	NP_000491.4	Autoimmune adrenalitis
Steroid 17-alpha-hydroxylase/17,20 lyase	NP_000093.1	Autoimmune adrenalitis
Potassium-transporting ATPase alpha chain	AAB50172.1	Autoimmune gastritis
Potassium-transporting ATPase beta chain	AAA35987.1	Autoimmune gastritis
Cytochrome P450 2D6	ABB77909.1	Autoimmune hepatitis
Genome polyprotein	S35630	Autoimmune hepatitis
O-phosphoserine-tRNA(Sec) selenium transferase	NP_058651.3	Autoimmune hepatitis
Asialoglycoprotein receptor	AAB58308.1	Autoimmune hepatitis
Glutathione S-transferase	CAA48637.1	Autoimmune hepatitis
Cytokeratin 8	AAB18966.1	Autoimmune hepatitis
M protein	AAA26918.1	Autoimmune myocarditis
Myosin-7	NP_000248.2	Autoimmune myocarditis
Cardiac myosin light chain 1	AAF91089.1	Autoimmune myocarditis
Cardiac myosin light chain 2	AAA91832.1	Autoimmune myocarditis
Cardiac actin	NP_005150.1	Autoimmune myocarditis
Troponin I	AC14461.1	Autoimmune myocarditis
Thyroid peroxidase	AAA61217.2	Autoimmune thyroiditis
Thyrotropin receptor	AAB23390.2	Autoimmune thyroiditis
Thyroglobulin	NP_003226.4	Autoimmune thyroiditis
S-arrestin	NP_000532.2	Autoimmune uveitis
LAMP2	AAB67314.1	Autoimmune vasculitis
Myeloperoxidase	AAA59863.1	Autoimmune vasculitis

Myeloblastin	NP_002768.3	Autoimmune vasculitis
Alpha-gliadin	AFX69628.1	Coeliac disease
Protein-glutamine gamma-glutamyltransferase 2	NP_004604.2	Coeliac disease
75k gamma secalin	ADP95479.1	Coeliac disease
Gamma I hordein	AFM77738.1	Coeliac disease
Avenin-3-like	ADA62372.1	Coeliac disease
Glycosyltransferase	ANR93567.1	Crohn's disease
60 kDa heat shock protein, mitochondrial	NP_002147.2	Crohn's disease
Transmembrane protein UO-44D	NP_002147.2	Crohn's disease
GM-CSF	AAA52578.1	Crohn's disease
Sucrase-isomaltase, intestinal	NP_001032.2	Crohn's disease
Glutathione peroxidase 2	NP_002074.2	Crohn's disease
60 kDa chaperonin 2	ARX70571.1	Crohn's disease
Pancreatic secretory glycoprotein 2	NP_001493.2	Crohn's disease
60 kDa chaperonin 2	OMH58317.1	Crohn's disease
Cytoskeleton-associated protein 5	EAW67976.1	Crohn's disease
AhpC	ETZ42359.1	Crohn's disease
Leukotriene B4 receptor 2	NP_062813.2	Crohn's disease
Chromodomain-helicase-DNA-binding protein 4	NP_001264.2	Dermatomyositis
Chromodomain-helicase-DNA-binding protein 3	NP_001005273.1	Dermatomyositis
Beta-1 adrenergic receptor	NP_000675.1	Dilated cardiomyopathy
Muscarinic acetylcholine receptor M2	NP_001006633.1	Dilated cardiomyopathy
Collagen alpha-3(IV) chain	CAA56335.1	Goodpasture's syndrome
Thyrotropin receptor	AAB23390.2	Grave's disease
Thyroid peroxidase	AAA61217.2	Grave's disease
Thyroglobulin	CAA29104.1	Grave's disease
Glutamate decarboxylase 2	NP_000809.1	Grave's disease
TSHR protein	AAI27629.1	Grave's disease
Thyroid peroxidase	AAA61217.2	Hashimoto's thyroiditis
Thyroglobulin	CAA29104.1	Hashimoto's thyroiditis
Thyroid stimulating hormone receptor	AAI41971.1	Hashimoto's thyroiditis
Insulin	AAA59172.1	Hypoglycemia
Insulin receptor	AAA59452.1	Hypoglycemia
Integrin beta-3	NP_000203.2	Immune thrombocytopenic purpura
Integrin alpha-IIb	NP_000410.2	Immune thrombocytopenic purpura
Platelet glycoprotein Ib alpha chain	NP_000164.5	Immune thrombocytopenic purpura

Platelet glycoprotein IIIa	AAA52600.1	Immune thrombocytopenic purpura
Thrombopoietin	AAB03393.1	Immune thrombocytopenic purpura
Insulin receptor	AAA59452.1	Insulin resistant diabetes
Phospholipase A2	NP_000919.1	Membranous nephritis
Myelin basic protein	AAC41944.1	Multiple sclerosis
Myelin proteolipid protein	AAA59565.1	Multiple sclerosis
Myelin-oligodendrocyte glycoprotein	CAA52617.1	Multiple sclerosis
Epstein-Barr nuclear antigen 1	Q1HVF7.1	Multiple sclerosis
DNA polymerase catalytic subunit	AMD82168.1	Multiple sclerosis
2',3'-cyclic-nucleotide 3'-phosphodiesterase	AAB24298.2	Multiple sclerosis
Oligodendrocyte-myelin glycoprotein	AAA59970.1	Multiple sclerosis
Aquaporin-4	AAH22286.1	Multiple sclerosis
Actin, cytoplasmic 1	NP_001092.1	Multiple sclerosis
Transposase, mutator family protein	EUA40098.1	Multiple sclerosis
E4 gene product	YP_002640224.1	Multiple sclerosis
Protein BOLFI	AIE89051.1	Multiple sclerosis
Myelin-associated glycoprotein	AAH93045.1	Multiple sclerosis
Transaldolase	NP_006746.1	Multiple sclerosis
Possible transposase	CCP46656.1	Multiple sclerosis
Claudin-11	NP_005593.2	Multiple sclerosis
Interferon beta	AAC41702.1	Multiple sclerosis
Alpha-crystallin B chain	ACA05949.1	Multiple sclerosis
Apolipoprotein E	AAB59518.1	Multiple sclerosis
Epstein-Barr nuclear antigen 6	AAA45895.1	Multiple sclerosis
Trans-activator protein BZLF1	BAP94413.1	Multiple sclerosis
Hemagglutinin	ALB07770.1	Multiple sclerosis
Protein S100-B	NP_006263.1	Multiple sclerosis
DNA polymerase catalytic subunit	SCL76875.1	Multiple sclerosis
Tripartite terminase subunit UL15	SCL76864.1	Multiple sclerosis
Glyceraldehyde-3-phosphate dehydrogenase	CAA25833.1	Multiple sclerosis
Alpha-enolase	CAA34360.1	Multiple sclerosis
Neurofilament light polypeptide	NP_006149.2	Multiple sclerosis
Connexin 43	AAA52131.1	Multiple sclerosis
Neurofilament medium polypeptide	NP_005373.2	Multiple sclerosis
POTE ankyrin domain family member I	NP_001264335.1	Multiple sclerosis
60 kDa heat shock protein, mitochondrial	NP_002147.2	Multiple sclerosis
Epstein-Barr nuclear antigen 3	BAP94411.1	Multiple sclerosis

Putative HTLV-1-related endogenous sequence	CAA34646.1	Multiple sclerosis
Glial fibrillary acidic protein	AAB22581.1	Multiple sclerosis
Phosphomannomutase/phosphoglucomutase	OPA62825.1	Multiple sclerosis
Minor capsid protein L2	P36745.1	Multiple sclerosis
N-acetylmuramoyl-L-alanine amidase CwlH	KIX84070.1	Multiple sclerosis
ATP-sensitive inward rectifier potassium channel 10	NP_002232.2	Multiple sclerosis
mRNA export factor ICP27 homolog	YP_401659.1	Multiple sclerosis
Acetylcholine receptor subunit alpha	NP_001034612.1	Myasthenia gravis
Acetylcholine receptor subunit gamma	NP_005190.4	Myasthenia gravis
Acetylcholine receptor subunit delta	NP_000742.1	Myasthenia gravis
Acetylcholine receptor subunit epsilon	NP_000071.1	Myasthenia gravis
Muscarinic receptor	AAB95158.1	Myasthenia gravis-MUSC
Aquaporin 4	AAH22286.1	Neuromyelitis optica
Alpha-synuclein	NP_000336.1	Parkinson's disease
DNA polymerase processivity factor	SBO07788.1	Parkinson's disease
Desmoglein-3	NP_001935.2	Phemphigus
Collagen alpha-1(XVII) chain	NP_000485.3	Phemphigus
Desmoglein-1	NP_001933.2	Phemphigus
Glutamate decarboxylase 2	NP_000809.1	Prediabetes
60 kDa heat shock protein, mitochondrial	NP_002147.2	Prediabetes
Insulin	AAA59172.1	Prediabetes
Insulin, isoform 2	NP_001035835.1	Prediabetes
Islet cell antigen	NP_002837.1	Prediabetes
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	NP_001922.2	Primary biliary cirrhosis
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	OAF98393.1	Primary biliary cirrhosis
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	WP_032229692.1	Primary biliary cirrhosis
Glycogen phosphorylase	AAC18079.1	Primary biliary cirrhosis
Nuclear pore glycoprotein 210	NP_079199.2	Primary biliary cirrhosis
Sarcosine dehydrogenase	AAD32214.1	Primary biliary cirrhosis
Sulfite oxidase	AAA74886.1	Primary biliary cirrhosis
Transglutaminase	BAA14329.1	Primary biliary cirrhosis
Nuclear autoantigen Sp-100	NP_001073860.1	Primary biliary cirrhosis

Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial	NP_001924.2	Primary biliary cirrhosis
Nuclear pore p62	AAA59990.1	Primary biliary cirrhosis
M protein precursor	AAA26918.1	Psoriasis
Keratin, type I cytoskeletal 16	NP_005548.2	Psoriasis
Keratin, type I cytoskeletal 17	NP_000413.1	Psoriasis
ADAMTS-like protein 5	NP_998769.2	Psoriasis
Transcriptional activator	AHF70996.1	Psoriatic arthritis
Fibrinogen alpha chain	AAI01936.1	Psoriatic arthritis
Vimentin	NP_003371.2	Psoriatic arthritis
Nebulin-related-anchoring protein	AI26408.1	Psoriatic arthritis
M protein	CAM31002.1	Rheumatic fever
Myosin-2	NP_060004.3	Rheumatic fever
Fibrinogen beta chain	AAI06761.1	Rheumatoid arthritis
Vimentin	NP_003371.2	Rheumatoid arthritis
Rheumatoid factor (IgG)	AAH73766.1	Rheumatoid arthritis
Glucose-6-phosphate isomerase	ARJ36701.1	Rheumatoid arthritis
Collagen alpha-1(II) chain	NP_001835.3	Rheumatoid arthritis
Fibrinogen alpha chain	AAI01936.1	Rheumatoid arthritis
Alpha-enolase	CAA34360.1	Rheumatoid arthritis
Tryptase precursor	AAA86934.1	Rheumatoid arthritis
Filaggrin	NP_002007.1	Rheumatoid arthritis
Aggrecan core protein	NP_001126.3	Rheumatoid arthritis
Small nuclear ribonucleoprotein Sm D1	NP_008869.1	Rheumatoid arthritis
Ribosomal protein L23a	AAB17510.1	Rheumatoid arthritis
60 kDa chaperonin 2	OMH58317.1	Rheumatoid arthritis
Trans-activator protein BZLF1	BAP94413.1	Rheumatoid arthritis
Epstein-Barr nuclear antigen 1	YP_401677.1	Rheumatoid arthritis
Chaperone protein DnaJ	EDV64758.1	Rheumatoid arthritis
60 kDa heat shock protein, mitochondrial	NP_002147.2	Rheumatoid arthritis
Chitinase-3-like protein 1	NP_001267.2	Rheumatoid arthritis
mRNA export factor ICP27 homolog	YP_401659.1	Rheumatoid arthritis
Arrestin	AAC50992.1	Rheumatoid arthritis, iritis
Protein BOLFI	AIE89051.1	Rheumatoid arthritis, juvenile
60 kDa heat shock protein, mitochondrial	NP_002147.2	Rheumatoid arthritis, juvenile
Major DNA-binding protein	BAX36606.1	Rheumatoid arthritis, juvenile
Keratin, type II cytoskeletal 3	NP_476429.2	Rheumatoid arthritis, juvenile
Fibrillin 1	BAD16739.1	Rheumatoid arthritis, juvenile
Tenascin precursor	NP_002151.2	Rheumatoid arthritis, juvenile

Stromelysin-1 preproprotein	NP_002413.1	Rheumatoid arthritis, juvenile
Interstitial collagenase	NP_002412.1	Rheumatoid arthritis, juvenile
OspA	CAA32579.1	Rheumatoid arthritis, Lyme
Integrin alpha-L	NP_002200.2	Rheumatoid arthritis, Lyme
DNA topoisomerase 1	NP_003277.1	Scleroderma/Systemic sclerosis
Histone H3-like centromeric protein A	NP_001800.1	Scleroderma/Systemic sclerosis
Small nuclear ribonucleoprotein Sm D1	NP_008869.1	Scleroderma/Systemic sclerosis
Major centromere autoantigen B	NP_001801.1	Scleroderma/Systemic sclerosis
E3 ubiquitin-protein ligase TRIM21	NP_003132.2	Scleroderma/Systemic sclerosis
Epstein-Barr nuclear antigen 1	YP_401677.1	Scleroderma/Systemic sclerosis
U11/U12 snRNP	Q6IEG0	Scleroderma/Systemic sclerosis
rRNA 2'-O-methyltransferase fibrillarin	NP_001427.2	Scleroderma/Systemic sclerosis
Ribonuclease P protein subunit p25	NP_060263.2	Scleroderma/Systemic sclerosis
60 kDa SS-A/Ro ribonucleoprotein	NP_001166995.1	Sjogren's syndrome
Lupus La protein	NP_003133.1	Sjogren's syndrome
E3 ubiquitin-protein ligase TRIM21	NP_003132.2	Sjogren's syndrome
Muscarinic acetylcholine receptor M3	NP_000731.1	Sjogren's syndrome
Small nuclear ribonucleoprotein Sm D1	NP_008869.1	Sjogren's syndrome
U1 small nuclear ribonucleoprotein A	NP_004587.1	Sjogren's syndrome
Putative HTLV-1-related endogenous sequence	CAA34646.1	Sjogren's syndrome
Calreticulin	AAB51176.1	Sjogren's syndrome
Spectrin alpha chain, non-erythrocytic 1	NP_001123910.1	Sjogren's syndrome
Beta-tubulin	AAB59507.1	Sydenham's chorea
Dopamine receptor 1	NP_000785.1	Sydenham's chorea
Dopamine receptor 2	NP_000786.1	Sydenham's chorea
60 kDa SS-A/Ro ribonucleoprotein	NP_001166995.1	Systemic lupus erythematosus
Small nuclear ribonucleoprotein Sm D1	NP_008869.1	Systemic lupus erythematosus
U1 small nuclear ribonucleoprotein 70 kDa	NP_003080.2	Systemic lupus erythematosus
Natural killer group protein 2-A	AAC17488.1	Systemic lupus erythematosus
Small nuclear ribonucleoprotein-associated proteins B and B'	NP_937859.1	Systemic lupus erythematosus
Small nuclear ribonucleoprotein-associated protein N	NP_001336393.1	Systemic lupus erythematosus
E3 ubiquitin-protein ligase TRIM21	NP_003132.2	Systemic lupus erythematosus
Epstein-Barr nuclear antigen 1	YP_401677.1	Systemic lupus erythematosus
U1 small nuclear ribonucleoprotein C	NP_003084.1	Systemic lupus erythematosus
NHP2-like protein 1	NP_001003796	Systemic lupus erythematosus
60S acidic ribosomal protein P2	XP_805182.1	Systemic lupus erythematosus

Histone H1.4	NP_005312.1	Systemic lupus erythematosus
Glutamate decarboxylase 2	NP_000809.1	Type 1 diabetes
Insulin	AAA59172.1	Type 1 diabetes
Islet cell antigen	NP_002837.1	Type 1 diabetes
Glucose-6-phosphatase 2	NP_066999.1	Type 1 diabetes
60 kDa heat shock protein, mitochondrial	AAH02676.1	Type 1 diabetes
Zinc transporter 8	AAP44332.1	Type 1 diabetes
Insulin, isoform 2	NP_001035835.1	Type 1 diabetes
Genome polyprotein	AAX23962.1	Type 1 diabetes
Islet amyloid polypeptide	NP_000406.1	Type 1 diabetes
Hemagglutinin	ALB07770.1	Type 1 diabetes
Islet amyloid polypeptide	NP_000406.1	Type 2 diabetes
Zinc transporter 8	AAP44332.1	Type 2 diabetes
Pancreatic secretory glycoprotein 2	NP_001493.2	Ulcerative colitis
GM-CSF	AAA52578.1	Ulcerative colitis
Myeloblastin	NP_002768.3	Ulcerative colitis
Type VII collagen	AAA96439.1	Ulcerative colitis
Melanocyte protein PMEL	NP_001186983.1	Vitiligo
Melanin-concentrating hormone receptor 1	NP_005288.3	Vitiligo
Tyrosine 3-monooxygenase	NP_954986.2	Vitiligo
Tyrosinase	NP_000363.1	Vitiligo
L-dopachrome tautomerase	NP_001913.2	Vitiligo
TrpC1	NP_001238774.1	Vitiligo
Myeloblastin	NP_002768.3	Wegener's granulomatosis
Collagen alpha-1(II) chain	NP_001835.3	Wegener's granulomatosis

Table 2 below provides HLA and classes commonly associated with autoimmune disorders though in the exemplary case the HLA or a portion of the HLA will be patient specific and derived from high resolution sequence of the patient suffering from the disorder or a serological equivalent.

Table 2. HLA types and classes commonly associated with autoimmune disease

Common HLA Class II Serotypes	Common Class II Variants	Common HLA Class I Serotypes	Common Class I Variants	Associated Autoimmune Disease
DR4 (e.g., DRB1*04); and DR5 (e.g., DRB1*11 and DRB1*12)	DRB1*04:01; DRB1*11; and DRB1*11:04	A2 (e.g., A*02)	A*02; A*02:01; and B*07:02	Alopecia areata
		A2		Alzheimer's
		B27 (e.g., B*2701-2759);	B*27:02; B*27:05;	Ankylosing spondylitis

		B40 (e.g., B*40); B27-B40; and B7 (e.g., B*07)	B*40:01; B*52; and B*38	
DR7 (e.g., DRB1*0701-0705); DR4; DR5; and DR12 (e.g., DRB1*1201-3 and DRB1*1206)	DRB1*09; DRB1*09:01; DRB1*04; DRB1*04:05; and DRB1*14			Antiphospholipid syndrome
DR17 (e.g., DRB1*0301 and DRB1*0304); DR4; DR4/DR3; DQ2 (e.g., DQB1*02); and DQ8 (e.g., DQB1*0302)	DRB1*03:01; DRB1*04; and DRB1*04:04			Autoimmune adrenalitis
DR2 (e.g., DRB1*15 and DRB1 DR4; DR5; DR2/DR4; and DR4/DR5				Autoimmune gastritis
	DRB1*03:01; DRB3*01:01; DRB1*04:01; DRB1*04:05; DRB1*07; and DRB1*13:01			Autoimmune hepatitis
DR7; DR4; DR11 (e.g., DRB1*1101 to DRB1*1110); DR3 (e.g., DRB1*03); and DR11-DQ7.5	DRB1*04:09; DRB1*07; and DRB1*04			Autoimmune myocarditis
		B27; A29 (e.g., A*29); and B51 (e.g., B*51)	A*29:02; and B*57:01	Autoimmune uveitis
DQ2; DQ8; DR12-DQ7.5; and DR7-DQ2.2	DQA1*05:01/ DQB1*02:01; DQA1*03/DQB1*03:02; and DQA1*0505/ DQBA1*0301			Celiac disease
DR1 (e.g., DRB1*01); and DR3	DRB1*07; DRB1*01:03; DRB1*0301; DRB1*0302; and DRB3*0301/DRB1*1302	B27		Crohn's disease

	DRB1*0301; and DRB1*0302			Dermatomyositis
DR4	DRB1*0302			Dilated cardiomyopathy
DR2		B8 (e.g., B*08)		Goodpasture's syndrome
DR17; DR52 (e.g., DRB3*); and DR7	DRB1*03:01; DRB1*04:01; DRB3*01; and DRB3*0202			Grave's disease
DR3; and DR5				Hashimoto's thyroiditis
DR4				Immune thrombocytopenic purpura
DR3; DR4; and DR3/DR4				Insulin resistant diabetes
DR3	DRB1*01:02			Membranous nephritis
DR2; DR15 (e.g., DRB1*1505-5 and DRB1*1507); and DR53 (DRB4*)	DRB1*15:01; DRB1*15:01/ DRB1*15:01; DRB1*15:01/ DRB5*01:01; DRB1*15; DRB5*01:01; DPw2; DRB1*04:01; DRB1*04:04; DPA1*01:03/ DPB1*02:01; DPA1*01:03/ DPB1*04:01; DQA1*01:02/ DQB1*05:02; DQB1*06; and DQB1*06:02	A3 (A*03); and B7		Multiple sclerosis
DR17; DR3; and DR7	DRB1*03:01			Myasthenia gravis
DR14-DQ5				Myasthenia gravis-MUSC
DR3				Neuromyelitis optica
DR patient specific				Parkinson's disease
DR4; and DR6	DRB1*01:01; and DRB1*04:02			Phemphigus
DR3; DR4; and DR3/DR4	DRB1*03:01; DRB1*04:01; and DRB1*03:01/* 04:01			Prediabetes
DR8 (e.g., DRB1*0801-*0807 and DRB1*0810- *0812)	DRB1*0801; and DRB1*0803			Primary biliary cirrhosis

DR7	DRB1*0102	B27; and Cw6 (C*06:02 and C*06:05)		Psoriasis
		B16 (e.g., B38 and B39); B17 (e.g., B57 and B58); B27; B39 (e.g., B*39); and Cw6		Psoriatic arthritis
DR7				Rheumatic fever
DR4; DR4-DQ8; DR1; DR12; and DR18 (e.g., DRB1*0302 and DRB1*0303)	DRB1*01:01; DRB1*01:02; DRB1*04:01; DRB1*04:02; DRB1*04:03; DRB1*04:04; DRB1*04:05; DRB1*04:06; DRB1*04:07; DRB1*04:08; DRB1*04:09; DRB1*04:10; DRB1*04:11; DRB1*04:12; DRB1*04:13; homozygous for the above;; DRB1*01:01/*04:04; and DRB1*01:01/*04:01			Rheumatoid arthritis
DR4; DR5; DR14 (e.g., DRB1*1401-*1408 and DRB1*1410-*1408); and DR15	DRB1*04:01; DRB1*04:04; DRB1*04:05; DRB1*14:02; and DRB1*12:01			Rheumatoid arthritis, juvenile
DR5; and DR11	DRB1*04:01; DRB1*10:01; and DRB1*11:02			Rheumatoid arthritis, Lyme
DR11; and DR8	DRB1*11:04			Rheumatoid arthritis, pauciarticular (juvenile)
DR5	DRB1*12:01	B35		Rheumatoid arthritis, iritis
DR5	DQB1*05:01; DRB1*11; DRB1*11:04; DRB1*15:02; DRB1*13:02; DRB1*04:06;			Scleroderma/Systemic sclerosis
	DRB1*03; DRB1*15;			Sjogren's syndrome

	DRB1*03:01/ DRB1*15:01			
DR1		B49 (e.g., B*49)		Sydenham's chorea
DR11; and DR53- DR7	DRB1*03:01; DRB1*15:01; DRB1*04:02; DRB1*04:03; DRB1*04:06; DRB1*11:01; and DRB3*03:01			Systemic lupus erythematosus
DR3	DRB1*03:02; DRB1*04; DRB1*04:01; DRB1*04:02; DRB1*04:05; DRB1*03:01; and DRB1*03:01/ DRB1*04:01			Type 1 diabetes
DR4				Type 2 diabetes
DR1	DRB1*01:03; and DRB1*15:02	B27		Ulcerative colitis
	DRB1*07:01		A*02:01	Vitiligo
	DPB1*04			Wegener's granulomatosis

In some embodiments, the antigenic peptides used herein are associated with HLA-DR*1501, for example, GAD peptide TYEIPVVFVLLFYVTLKKMR (SEQ ID NO: 34) (involved in Type I diabetes), the MBP peptides listed above, the following MPP peptides (involved in MS) LLECCARCLVGAPFASLVATGLCFFGVAF (SEQ ID NO: 35), LVGAPFASLVATGLCFFGVA (SEQ ID NO: 36), FGVALFCGCEVEALTGTEKLIETYFSKNYQD (SEQ ID NO: 37), LFCGCGHEALTGTEKLIETY (SEQ ID NO: 38), TGTEKLIETYFSKNYQDYEY (SEQ ID NO: 39), TGTEKLIETYFSKNYQDYEYL (SEQ ID NO: 40), YFSKNYQDYEYLVINVIHAFQYVIYGTASFFFL (SEQ ID NO: 41), GTASFFFLYGALLLAYGFYTTGAVRQIFGDYK (SEQ ID NO: 42), LYGALLAEGFYTTGAVRQI (SEQ ID NO: 43), FYYTTGAVRQIFGDYKTTICG (SEQ ID NO: 44), AVRQIFGDYKTTICGKGLSATV (SEQ ID NO: 45), RQIFGDYKTTICGKGLSATVTGGQKGRGSRGQ (SEQ ID NO: 46), KGLSATVTGGQKGRGYRGQH (SEQ ID NO: 47),

QKGRGSRGQHQAHS�ERVCH (SEQ ID NO: 48),
 KGRGSRGQHQAHS�ERVCHCLGCLWLGHPDKFV (SEQ ID NO: 49),
 LGHPDKFVGITYALTVVWLLVFACSAVPVYIY (SEQ ID NO: 50),
 SAVPVYIYFNTWTTTCQSIAAPCKTSASIGTLC (SEQ ID NO: 51),
 AVPVYIYFNTWTTTCQSIAFP (SEQ ID NO: 52),
 WTTTCQSIAFPSKTSASIGSL (SEQ ID NO: 53),
 SASIGTLCADARMYGVLPWNAFFGKVCGSNLL (SEQ ID NO: 54),
 KVCGSNLLSICKTAEFQMTFHLFIAAFVGAAA (SEQ ID NO: 55),
 AAFVGAAATLVSLLTFMIAATYNFAVLKLMGR (SEQ ID NO: 56),
 MIAATYNFAVLKLMGRGTKF (SEQ ID NO: 57), and
 MAATYNFAVLKLMGRFTKF (SEQ ID NO: 58).

In some embodiments, the antigenic peptides or antigenic polypeptides are patient specific and designed for the patient's MHC. For example, a physician can diagnose the patient with an autoimmune disorder and determine the severity of the disease. The patient's Class I (HLA-A, B, and C) and II (HLA-DR, DQ, DP) regions can be typed, which can now be performed at high resolution using DNA sequencing and with comparison to a reference database (www.ebi.ac.uk/ipd/imgt/hla/). The patient's Class I and II MHC with the strongest evidence of autoimmune involvement can be identified for the disorder. Those known to be associated with a particular autoimmune disorder can be used as references. See, *e.g.*, Tables 1 and 2. The strongest evidence based antigens are identified for the disorder (iedb.org/) and Table 1. A set of personalized peptide (cbs.dtu.dk/services/NetMHC/ or cbs.dtu.dk/services/NetMHCII/) and protein targets (for Class II) that are expected to bind the patient autoimmune implicated MHC can be identified.

Personalized MHC-CARs lentivirus or mRNA can be prepared for the patient to enable targeting of pathogenic immune cells. The personalized lentivirus is used to prepare autologous or allogeneic T cells (CTL and/or Tregs) that can be combined with receptor or cellular modifications to allow co-treatment with additional therapeutics, desired interactions with pathogenic cells, routing to a desired location (for interaction with pathogenic inflammatory or inflammation generating cells), or secretion of cytokines (to reduce inflammation).

(c) Co-stimulatory signaling domains

Many immune cells require co-stimulation, in addition to stimulation of an antigen-specific signal, to promote cell proliferation, differentiation and survival, as well as to

activate effector functions of the cell. The MHC-CAR described herein may comprise one or more co-stimulatory signaling domain. The term “co-stimulatory signaling domain,” as used herein, refers to at least a portion of a protein that mediates signal transduction within a cell to induce an immune response such as an effector function. The co-stimulatory signaling domain of the MHC-CAR described herein can be a cytoplasmic signaling domain from a co-stimulatory protein, which transduces a signal and modulates responses mediated by immune cells, such as T cells, NK cells, macrophages, neutrophils, or eosinophils.

Activation of a co-stimulatory signaling domain in a host cell (*e.g.*, an immune cell) may induce the cell to increase or decrease the production and secretion of cytokines, phagocytic properties, proliferation, differentiation, survival, and/or cytotoxicity. The co-stimulatory signaling domain of any co-stimulatory molecule may be compatible for use in the MHC-CAR described herein. Examples of co-stimulatory signaling domains for use in the chimeric receptors can be the cytoplasmic signaling domain of co-stimulatory proteins, including, without limitation, members of the B7/CD28 family (*e.g.*, B7-1/CD80, B7-2/CD86, B7-H1/PD-L1, B7-H2, CD28, CTLA-4, ICOS/CD278, or PD-1); members of the TNF superfamily (*e.g.*, 4-1BB/TNFSF9/CD137, 4-1BB Ligand/TNFSF9, CD40/TNFRSF5, CD40 Ligand/TNFSF5, DR3/TNFRSF25, OX40/TNFRSF4, OX40 Ligand/TNFSF4, or TNF- α); and other molecules, such as FRB, and FKBF, that allow co-stimulation to be induced only in the presence of a specific drug molecule (but here in association with a the unique heterodimeric MHC-CAR). Wu et al., *Science*, 350(6258):aab4077, 2015. In some embodiments, any of the cytoplasmic signaling domains of co-stimulatory proteins may be used in receptors targeting inactive bystander B cells (*e.g.*, with a CD19 or CD20-CAR) or plasma cells (*e.g.*, with a CS1-CAR and/or CS1 knockout).

In some instances, the MHC-CAR may comprise a combination (*e.g.*, 2 or 3) co-stimulatory domains, which may be from the same co-stimulatory receptor or from different co-stimulatory receptors. Examples include: CD28+4-1BB, CD28+FRB, CD28+FKBF, or 4-1BB+FRB. See also Figure 9. In some examples, the MHC-CAR comprises a co-stimulatory domain from CD28, a co-stimulatory domain from 4-1BB, or both. In some embodiments, the co-stimulatory domain is preceded by a short linker. For example, for a class II MHC-CAR, the short linker may be TS (*i.e.*, a MHC internal Linker); for a class I MHC-CAR, the short linker may be PG.

In some instances, the MHC-CAR constructs described herein may include no co-stimulatory domain. Alternatively, it may contain a non-traditional element such as a TALEN nuclease, activators, or repressors which may now be implemented in a clinically applicable lentiviral form using a recoded or non-repeat containing TAL domain and would be linked to a single chain MHC-CAR through a membrane domain derived from Notch.

Exemplary co-stimulatory domains for use in the MHC-CAR described herein include, but are not limited to:

41BB intracellular domain: KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 59)

41BB_e intracellular domain: pgKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELaha (SEQ ID NO: 60)

CD28 intracellular domain: RSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS (SEQ ID NO: 61)

CD28_e intracellular domain: pgRSKRSRGGHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSaha (SEQ ID NO: 62)

FRB:

EMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKD
LLQAWDLYYHVFRI (SEQ ID NO: 63)

FBP-with linkers

(GSSS)₄-
EMWHEGLEEASRLYFGERNVKGMEFVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNV
KDLLQAWDLYYHVFRI-(GSSS)₃ (SEQ ID NO: 64)

FKRB

GVQVETISPGDGRTPFKRGQTCVVHYTGMLEDGKKFDSSRDNRNKPFFKMLGKQEVIRGWEEGVAQMSVGQRAKLT
ISPDYAYGATGHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 65)

FKBP-with linkers

(GSSS)₄-
GVQVETISPGDGRTPFKRGQTCVVHYTGMLEDGKKFDSSRDNRNKPFFKMLGKQEVIRGWEEGVAQMSVG
QRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLE-(GSSS)₃ (SEQ ID NO: 66)

(d) Cytoplasmic signaling domain

Any cytoplasmic signaling domain comprising an immunoreceptor tyrosine-based activation motif (ITAM) can be used to construct the chimeric receptors described herein.

An "ITAM," as used herein, is a conserved protein motif that is generally present in the tail portion of signaling molecules expressed in many immune cells. The motif may comprises two repeats of the amino acid sequence YxxL/I separated by 6-8 amino acids, wherein each x is independently any amino acid, producing the conserved motif YxxL/Ix(6-8)YxxL/I. In some examples, the cytoplasmic signaling domain comprising an ITAM is of CD3 ζ . In some examples, the MHC-CAR does not comprise a co-stimulatory domain and the cytoplasmic signaling domain is preceded by a short linker. For example, for a class II MHC-CAR, the short linker may be TS (*i.e.*, a MHC internal Linker). For example for a class I MHC-CAR, the short linker may be PG. In some cases the linker may be AHA or absent, such as certain instances when a co-stimulatory domain occurs before a signaling domain.

In some embodiments, the MHC-CAR may include no cytoplasmic signaling domain, for example, that of CD3 ζ . Such CD3 ζ -free MHC-CAR would have suppressive effects against target cells or induce target cell death. Moisini, et al., The Journal of Immunology, 180(5), pp.3601-3611.

Provided below is an exemplary cytoplasmic signaling domain from CD3 ζ :

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPFEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 67)

Provided below are exemplary nucleic acid sequences encoding a cytoplasmic signaling domain from CD3 ζ :

AGAGTAAAGTTTTCCTCGAAGTGCAGGACGCTCCCGCGTATCAGCAAGGTCAAAACCAAGCTTTACAACGAACCTGAAC
TTGGGACGACGCGAAGAGTACGATGTTCTTGATAAGCGGAGAGGGCGCGATCCCGAAATGGGGGAAAGCCTCGG
AGGAAGAACCACAAGAAGGCCCTTTATAATGAACTGCAGAAGGACAAGATGGCGGAGGCGTATTCGAAATAGGC
ATGAAGGGTGAACGGAGGAGAGGAAAGGGACATGACGGACTTTATCAAGGATTGTCTACCGCAACTAAAGACACC
TATGACGCGTTGCACATGCAGGCTCTCCCTCCGAGA (SEQ ID NO: 410)

CGGGTCAAATTTAGCAGATCCGCTGACGCACCGGCTACCAGCAGGGCCAGAACCAACTCTACAACGAGCTGAAT
CTCGGCCGACGGGAAGAGTATGACGTACTCGACAAGCGGAGAGGTCGAGACCTGAGATGGGCGGTAAACCGAGA
CGGAAAAATCCCCAAGAGGGTCTTTATAATGAACTCCAGAAGGATAAGATGGCTGAAGCCTATTCTGAGATAGGG
ATGAAAGGCGAGCGCGGAGGGGTAAGGGCCATGATGGCCTTTACCAGGGACTCTCCACGGCAACCAAGATACT
TACGACGCCCTTCACATGCAAGCCCTCCCGCCACGC (SEQ ID NO: 422)

(e) Additional components

The MHC-CAR described herein may optionally further include one or more of the following components: a hinge domain, a transmembrane domain, a signal (leader) peptide, and a peptide linker.

In some instances, the antigenic peptide may be linked to a hinge peptide to enhance immune targeting activity of the resultant MHC-CAR and/or to reduce antibody responses by

the target cell to the MHC-TCR complex. In some examples, a MHC-CAR containing a hinge peptide may not include a cytoplasmic domain (for example, free of a CD3 ζ domain). A MHC-CAR construct that contains a hinge peptide may also include a MHC class I moiety. The hinge domain may contain about 10-100 amino acids, *e.g.*, 15-75 amino acids, 20-50 amino acids, or 30-60 amino acids. In some embodiments, the hinge domain may be of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, or 75 amino acids in length.

In some examples, the following peptide linkers can be used in a class I MHC-CAR:

MHCILinker 1: GGGGSGGGSGGGGS (SEQ ID NO: 68)

MHCILinker 2: GGGGSGGGSGGGSGG (SEQ ID NO: 69)

MHCILinker 3: GGGGSGGGSGGGSGGGGS (SEQ ID NO: 70)

MHCILinker 4: GGGGSGGGSGGGGS (SEQ ID NO: 68)

Exemplary peptide linkers for a class II MHC-CAR can be GSGSGSGS (MHCII LinkerI; SEQ ID NO: 72), GGGGSGGGSGGGGS (MHC II LinkerII; SEQ ID NO: 68), GGGGSGGGSGGS (SEQ ID NO: 400), or those described herein as MHC I Linkers (*i.e.*, MHC I Linkers 1-4). An exemplary pre-peptide linker for a class II MHC-CAR can be AS or GS or one or two copies of either AS or GS.

An example nucleic acid sequence encoding the peptide linker provided by SEQ ID NO: 400 is provided below

GGGGGAGGCGGATCTGGCGGAGGCGGGAGTGGAGGCTCA (SEQ ID NO: 401)

A hinge peptide for use in the MHC-CAR described herein may be derived from a naturally-occurring receptor. Hinge domains of any protein known in the art to comprise a hinge domain are compatible for use in the chimeric receptors described herein. In some embodiments, the hinge domain is a portion of the hinge domain of CD8 α , *e.g.*, a fragment containing at least 15 (*e.g.*, 20, 25, 30, 35, or 40) consecutive amino acids of the hinge domain of CD8 α . Alternatively, it may be a synthetic peptide.

Exemplary hinge domains include: IYIWAPLAGTCGVLLLSLVIT (SEQ ID NO: 73), and IWAPLAGICVALLLSLIITLI (SEQ ID NO: 74). Additional examples are provided below:

FKBP/FRB-CD8 hinge:

GVQVETISPGDGRTPFKRGQTCVVHYTGMLEDGKKFESSRDRNKPFKFM LGKQEVIRGWEEGVAQMSVGQRAKLT
ISPDYAYGATGHPGII PPATLVFDVELLKL EEAAREAAAREAAAREAAARGRVAILWHEMWHEGLEEASRLYF
GERNVKGMFEVLEPLHAMMERGPQTLKETSFNQAYGRDLMEAEWCRKYMKSGNVKDLLQAWDLYYHVFRRI TTT
PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 75)

GS short hinge: GGGGSGGGGSGGGGS (SEQ ID NO: 68)

GS long hinge: GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 76)

H2-Kb hinge: LRWEPPPSTVSNM (SEQ ID NO: 77)

HLA-A2 hinge: LRWEPSSQPTIPI (SEQ ID NO: 78)

HLA-A3 hinge: LRWELSSQPTIPI (SEQ ID NO: 79)

DAP10 hinge: QTPGERSSLP AFYPGTSGSCSGCSLSLP (SEQ ID NO: 80)

DAP10 hinge with linker: (GSSS)₄QTPGERSSLP AFYPGTSGSCSGCSLSLP (SEQ ID NO: 81)

DAP12 hinge: LRPVQAQAQSDCSCSTVSP (SEQ ID NO: 82)

DAP12 hinge with linker: (GSSS)₄LRPVQAQAQSDCSCSTVSP (SEQ ID NO: 83)

FcIgGIIIa hinge: GLAVSTISSEFFPPGYQ (SEQ ID NO: 84)

CD8α hinge: TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 85)

IgG1 hinge:

EPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDITLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTRP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT
CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMH EALHNHYTQKSL
SLSPGK (SEQ ID NO: 86)

DRA*0101 hinge: EFDAPSPLPETTE (SEQ ID NO: 87)

DRB1*1501 hinge: VEWRA RSESAQSK (SEQ ID NO: 88)

An example nucleic acid sequence encoding a DRA*0101 hinge is provided below.

GAGTTCGACGCCCCATCACCGCTTCCAGAAACGACTGAA (SEQ ID NO: 417)

An example nucleic acid sequence encoding a DRB1*1501 hinge is provided below.

GTTGAGTGGAGGGCGCGGTCAGAGAGCGCACAATCTAAA (SEQ ID NO: 404)

In some embodiments, the MHC-CAR constructs described herein further comprise a transmembrane domain. Any transmembrane domain for use in the MHC-

CAR can be in any form known in the art. As used herein, a “transmembrane domain” refers to any protein structure that is thermodynamically stable in a cell membrane, preferably a eukaryotic cell membrane. Transmembrane domains compatible for use in the chimeric receptors used herein may be obtained from a naturally-occurring protein. Alternatively, it can be a synthetic, non-naturally occurring protein segment, *e.g.*, a hydrophobic protein segment that is thermodynamically stable in a cell membrane.

Transmembrane domains are classified based on the three dimensional structure of the transmembrane domain. For example, transmembrane domains may form an alpha helix, a complex of more than one alpha helix, a beta-barrel, or any other stable structure capable of spanning the phospholipid bilayer of a cell. Furthermore, transmembrane domains may also or alternatively be classified based on the transmembrane domain topology, including the number of passes that the transmembrane domain makes across the membrane and the orientation of the protein. For example, single-pass membrane proteins cross the cell membrane once, and multi-pass membrane proteins cross the cell membrane at least twice (*e.g.*, 2, 3, 4, 5, 6, 7 or more times).

Membrane proteins may be defined as Type I, Type II or Type III depending upon the topology of their termini and membrane-passing segment(s) relative to the inside and outside of the cell. Type I membrane proteins have a single membrane-spanning region and are oriented such that the N-terminus of the protein is present on the extracellular side of the lipid bilayer of the cell and the C-terminus of the protein is present on the cytoplasmic side. Type II membrane proteins also have a single membrane-spanning region but are oriented such that the C-terminus of the protein is present on the extracellular side of the lipid bilayer of the cell and the N-terminus of the protein is present on the cytoplasmic side. Type III membrane proteins have multiple membrane-spanning segments and may be further sub-classified based on the number of transmembrane segments and the location of N- and C-termini.

In some embodiments, the transmembrane domain of the MHC-CAR described herein is derived from a Type I single-pass membrane protein, *e.g.*, CD8 α , CD8 β , 4-1BB/CD137, or CD28. Transmembrane domains from multi-pass membrane proteins may also be compatible for use in the chimeric receptors described herein. Multi-pass membrane proteins may comprise a complex (at least 2, 3, 4, 5, 6, 7 or more) alpha helices or a beta sheet structure. Preferably, the N-terminus and the C-terminus of a multi-pass membrane protein are present on opposing sides of the lipid bilayer, *e.g.*, the N-terminus

of the protein is present on the cytoplasmic side of the lipid bilayer and the C-terminus of the protein is present on the extracellular side. Either one or multiple helix passes from a multi-pass membrane protein can be used for constructing the chimeric receptor variant described herein.

Exemplary transmembrane domains for use in constructing the MHC-CAR constructs described herein are provided below:

CD8a transmembrane domain: IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 89)

HLA-A2 transmembrane domain: VGIIAGLVLF GAVITGAVVAAMW (SEQ ID NO: 90)

HLA-A3 transmembrane domain: VGIIAGLVLLGAVITGAVVAAMW (SEQ ID NO: 91)

Cd3zeta transmembrane domain: LCYLLDGILFIYGVILTALFL (SEQ ID NO: 92)

DR*1501 transmembrane domain: MLSGVGGEFVLGLLFLGAGLFI (SEQ ID NO: 93)

DR*1501e transmembrane domain: MLSGVGGEFVLGLLFLGAGLFIYFRNQ (SEQ ID NO: 94)

DRA*0101 transmembrane domain: NVVCALGLTVGLVGIIIGTIFII (SEQ ID NO: 416)

DRA*0101e transmembrane domain: NVVCALGLTVGLVGIIIGTIFIIRGL (SEQ ID NO: 418)

An example nucleic acid sequence encoding the DR*1501e transmembrane domain is provided below:

ATGCTGTCAGGAGTAGGCGGATTTGTACTCGGACTCCTCTTTTGGGCGCTGGGTGTTTATCTACTTTAGAAAC CAA (SEQ ID NO: 406)

An example nucleic acid sequence encoding the DRA*0101e transmembrane domain is provided below:

AACGTTGTCTGCGCTCTTGGCCTGACAGTGGGCTGGTAGGCATTATTATCGGGACCATCTTTATCATCAAAGGT TTG (SEQ ID NO: 419)

Notch transmembrane domain:

ILDYSFTGGAGRDIPPPQIEEACELPECQVDAGNKVCNLQCNNHACGWDGGDCSLNFNDPWKNCTQSLQ CWKYFSDGHCDSDQNSAGCLFDGFDCLTEGQCNPLYDQYCKDHFSDGHCDQGCNSAECEWDGLDCAEH VPERLAAGTLVLVLLPPDQLRNNSFHFLRELSHVLHTNVVFKRDAQGQQMIFPYYGHEEELRKHPKIR STVGWATSSLLPGTSGGRQRRELDPMDIRGSIVYLEIDNRQCVQSSSQCFQSATDVA AFLGALASLGSL NIPYKIEAVKSEPVPEPLPSQLHLMYAAAAFVLLFFVVGCGVLLSRKRRR (SEQ ID NO: 95)

Notch 2 transmembrane domain:

PCVGSNFCYNQGTCEPTSENPFYRCLCPAKFNGLLCHILDYSFTGGAGRDI PPPQIEEACELPECQVDA
 GNKVCNLQCNHACGWDGGDCSLNFNDPWKNCTQSLQCWKYFSDGHCDSCNSAGCLFDGFDQCQLTEGQ
 CNPLYDQYCKDHFSDGHCDQGCNSAECEWDGLDCAEHVPERLAAGTLVLVLLPPDQLRNNSFHFLREL
 SHVLHTNVVFKRDAQGQOMIFPYYGHEEELRKHPKIRSTVGWATSSLLPGTSGGRQRRELDPMDIRGSI
 VYLEIDNRQCQVSSSQCFQSATDVAAFLGALASLGSLNIPYKIEAVKSEPEPPLPSQLHLMYVAAAAF
 VLLFFVGCGLLSRKRRR (SEQ ID NO: 96)

In some embodiments, the MHC-CAR may also comprise a signal peptide (also known as a signal sequence or a leader peptide) at the N-terminus of the polypeptide. In general, signal sequences are peptide sequences that target a polypeptide to the desired site in a cell. In some embodiments, the signal sequence targets the MHC-CAR to the secretory pathway of the cell and will allow for integration and anchoring of the MHC-CAR into the lipid bilayer. Signal sequences including signal sequences of naturally occurring proteins or synthetic, non-naturally occurring signal sequences, that are compatible for use in the chimeric receptors described herein will be evident to one of skill in the art. In some embodiments, the signal sequence from CD8 α . In some embodiments, the signal sequence is from CD28 (*e.g.*, MLRLLLALNLFPSIQVTG (SEQ ID NO: 97)).

Exemplary signal peptides include, but are not limited to, Beta-2-microglobulin signal peptide (*e.g.*, MSRSVALAVLALLSLSGLEA (SEQ ID NO: 98)), HLA A3 signal peptide (*e.g.*, MAVMAPRTL LLLLSGALALTQTWA (SEQ ID NO: 99) or), DRA*0101 signal peptide (*e.g.*, MAISGVVPVLGFFIIAVLMSAQESWA (SEQ ID NO: 100)), DRB1* 1501 signal peptide (*e.g.*, MVCLKLPGGSCMTALTVTLMVLSSPLAL (SEQ ID NO: 101)), and DRB5 signal peptide (*e.g.*, MVCLKLPGGSYMAKLTVTLMVLSSPLALA (SEQ ID NO: 102)). Exemplary signal peptides may be followed by flexible pre-peptide linkers such as AS, GS, ASAS, GSGS. In some embodiments, a flexible pre-peptide linker is used when the signal peptide is class II and followed by an introduced peptide. Any of the constructs encoding the MHC-CARs described herein may comprise a nucleic acid sequence encoding any of the pre-peptide linkers above, *e.g.* AS may be encoded by the nucleic acid sequence GCATCT, TS may be encoded by the nucleic acid sequence ACAAGT.

Example nucleic acid sequence encoding beta-2-microglobulin signal peptides are provided below:

ATGGTATGCTTGAAGCTCCCGGGCGGGTCCTGCATGACCGCTCTCACTGTTACTCTTATGGTCCTTAGTTACCGG
 CTTGCCCTG (SEQ ID NO: 397)

ATGGCAATATCTGGTGTTCCTGTCTCGGGTTTTTATCATAGCCGTACTGATGTCAGCACAGGAATCATGGGCG
 (SEQ ID NO: 414)

In some embodiments, the MHC-CAR described herein may include one or more peptide linkers between the other components as described herein. Examples include a $(\text{Gly}_x\text{Ser})_n$ linker, wherein x and n , independently can be an integer between 3 and 12, including 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more. In some examples, the peptide linker can be $(\text{Gly}_4\text{Ser})_n$ (SEQ ID NO: 103), wherein n can be an integer between 3 and 20. Specific examples include $(\text{Gly}_4\text{Ser})_3$ (SEQ ID NO: 68), $(\text{Gly}_4\text{Ser})_6$ (SEQ ID NO: 69), $(\text{Gly}_4\text{Ser})_9$ (SEQ ID NO: 76), $(\text{Gly}_4\text{Ser})_{12}$ (SEQ ID NO: 105), and $(\text{Gly}_4\text{Ser})_{15}$ (SEQ ID NO: 106).

(ii) *Configuration of MHC-CARs*

The MHC-CAR constructs disclosed herein, comprising one or more components described herein, may be configured in any suitable format. Exemplary MHC class I constructs and MHC class II constructs are provided in Figures 7 and 8.

A MHC-CAR construct containing a MHC class I moiety as described herein may be a single fusion polypeptide that comprise the MHC class I moiety, the antigenic peptide, and a signaling domain (*e.g.*, a co-stimulatory domain, a cytoplasmic signaling domain, or a combination thereof), and optionally one or more of the additional components described herein. See, *e.g.*, Figure 8. In some examples, a MHC Class I CAR construct contains a hinge domain adjacent to the antigenic peptide. A MHC class I CAR may not contain $\beta 2$ -microglobulin (b2m). When expressed on cell surface, such a MHC-CAR may form a heterodimer with endogenous b2m. Alternatively, a MHC class I CAR may also include b2m, which may be fused with the alpha chain to produce a single polypeptide. In some instances, a MHC class I CAR may contain two subunits, one including the alpha chain or a portion thereof (*e.g.*, an extracellular domain), and the other including b2m or a portion thereof (*e.g.*, an extracellular domain). In some examples, the antigenic peptide may be fused to the alpha chain. In other examples, the antigenic peptide may be fused to b2m. Optionally, a MHC class I CAR may contain peptide linkers between two components. One example is provided in Figure 8B.

In some examples, the MHC-CAR comprises a class I molecule or a portion thereof, for example, HLA A3 or HLA A2, and an antigenic peptide suitable for presentation by the class I molecule (*e.g.*, the PLP fragment KLIETYFSK (SEQ ID NO: 107) or the TAX fragment LLFGYPVYV (SEQ ID NO: 108)). Optionally, the MHC-CAR may further comprise b2m. Alternatively, the b2m molecule may be expressed separately from the class I MHC-CAR. Examples of the class I molecules and b2m sequences are provided below:

HLA A2:

GSHSMRYFFTSVSRPGRGEPFRFIAVG YVDDTQFVRFDSDAASQRM EPRAPWIEQEGPEYWDGETRKVKAHSQTHR
 VDLGTLRGYYNQSEAGSHTVQRM YGCDVGS DWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQITKKHWEAA
 HVAEQLRAYLEGTCVEWLRRLYLENGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFP AEITLTWQRDGEDQT
 QDTELVETRPAGDGT FQKWA AVVVP SGQEQR YTCHVQH EGLPKPLT (SEQ ID NO: 109)

HLA A3:

GSHSMRYFFTSVSRPGRGEPFRFIAVG YVDDTQFVRFDSDAASQRM EPRAPWIEQEGPEYWDQETRN VKAQSQTDR
 VDLGTLRGYYNQSEAGSHTIQIMYGCDVGS DGRFLRGYRQDAYDGKDYIALNEDLRSWTAADMAAQITKKHWEAA
 HEAEQLRAYLDGTCVEWLRRLYLENGKETLQRTDPPKTHMTHHPISDHEATLRCWALGFYP AEITLTWQRDGEDQT
 QDTELVETRPAGDGT FQKWA AVVVP SGEEQR YTCHVQH EGLPKPLT (SEQ ID NO: 110)

Microextension for above: LRWE (SEQ ID NO: 111)

HLA A2 with H-2K^b alpha3 domain (underlined/italicized)

GSHSMRYFFTSVSRPGRGEPFRFIAVG YVDDTQFVRFDSDAASQRM EPRAPWIEQEGPEYWDGETRKVKAHSQTHR
 VDLGTLRGYYNQSEAGSHTVQRM YGCDVGS DWRFLRGYHQYAYDGKDYIALKEDLRSWTAADMAAQITKKHWEAA
 HVAEQLRAYLEGTCVEWLRRLYLENGKETLQRTDSPKAHVTHHSRPEDKVTLRCWALGFYPADITLTWQLNGEELI
QDMELVETRPAGDGT FQKWA SVVVP LGKEQYYTCHVYHQGLPEPLT (SEQ ID NO: 112)

HLA A3 with H-2K^b alpha3 domain (underlined/italicized)

GSHSMRYFFTSVSRPGRGEPFRFIAVG YVDDTQFVRFDSDAASQRM EPRAPWIEQEGPEYWDQETRN VKAQSQTDR
 VDLGTLRGYYNQSEAGSHTIQIMYGCDVGS DGRFLRGYRQDAYDGKDYIALNEDLRSWTAADMAAQITKKHWEAA
 HEAEQLRAYLDGTCVEWLRRLYLENGKETLQRTDSPKAHVTHHSRPEDKVTLRCWALGFYPADITLTWQLNGEELI
QDMELVETRPAGDGT FQKWA SVVVP LGKEQYYTCHVYHQGLPEPLT (SEQ ID NO: 113)

Microextension for above: LRWE (SEQ ID NO: 111)

Beta-2-microglobulin (human):

IQRTPKIQVYSRHPAENGKSNFLN CYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTTEK
 DEYACRVNHVTLSQPKIVKWRDM (SEQ ID NO: 114)

Beta-2-microglobulin (mouse):

IQKTPQIQVYSRHPPENGKPNILN CYVTQFHPPHIEIQMLKNGKKIPKVEMSDMSFSKDWSFYILAHTTEFTPTET
 DTYACRVKHA S MAEPKTVYWRDM (SEQ ID NO: 115)

Figure 7 provides a number of exemplary designs of MHC class II CAR constructs. Typically, a MHC class II CAR construct contains two subunits, one including the alpha chain or a portion thereof (e.g., an extracellular domain) and the other including the beta chain or a portion thereof (e.g., an extracellular domain). The antigenic peptide can be fused to either the alpha chain or the beta chain. In some instances, a MHC class II CAR can also be in a single fusion polypeptide format, in which the alpha and beta chains are fused to form

a single polypeptide. The alpha chain and beta chain of a MHC class II CAR may be derived from the same MHC class II molecule. Alternatively, they may be from different MHC class II molecules. For example, a MHC class II CAR may contain an alpha chain from HLA DRA*1010 and a beta chain from HLA DRB1*1501, which may be fused with an antigenic peptide, such as an MBP peptide.

In some examples, the MHC-CAR comprises a class II molecule or a portion thereof, for example, DRB1*1501 or DRA*0101, and an antigenic peptide suitable for presentation by the class II molecule (*e.g.*, the MBP fragment DENPVVHFFKNIVTPRTPP (SEQ ID NO: 15)). Examples of the class II molecule sequences are provided below;

DRB1*1501:

GDTRPRFLWQPKRECHFFNGTERVRFLDRYFYNQEESSVRFDSDVGEFRAVTELGRPDAEYWNSQKDILEQARAADV
DTYCRHNYGVVESFTVQRRVQPKVTVPYPSKTQPLQHHNLLVCSVSGFYPGSIEVRWFNLNGQEEKAGMVSTGLIQN
GDWTFQTLVMLETVPRSGEVYTCQVEHPSVTSPLT (SEQ ID NO :117)

DRA*0101

IKEEHVILQAEFYLNPDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTK
RSNYTPITNVPPEVTVLNTPVELREPNVLICFIDKETPPVVNVTLWRNGKPVTTGVSETVFLPREDHLERKFHY
LPFLPSTEDVYDCRVEHWGLDEPLLKHW (SEQ ID NO: 118)

DRB1*1501 human/IA-Dbeta mouse (mutated residues in boldface and underlined)

IKEEHVILQAE**S**YLNPDQSGEF**K**FDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTK
RSNYTPLEETEVPPTSLRRLQPNVAISLSRTEALNHHNTLVCSVTDFYPAKIKVRWFRNGQEETVGVSSSTQLIRN
GDWTFQVLVMLEMTPHQGEVYTCHVEHPSLKSPIT (SEQ ID NO: 119)

DRA*0101 human/IA-Dalpha mouse (mutated residues in boldface and underlined)

IKEEHVILQAE**S**YLNPDQSGEF**K**FDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTK
RSNYTPITATNEAPQATVFPKSPVLLGQPHTLICFVDNIFPPVINITWLRNSKSVTDGVYETSFLVNRDHSFHKLS
YLTF
IPSDDDIYDCKVEHWGLEEPVLKHWEPEI (SEQ ID NO: 120)

DR-2_{beta} mini (mutated residue in boldface and underlined)

RPRFLWQ**S**KRECHFFNGTERVRFLDRYFYNQEESSVRFDSDVGEFRAVTELGRPDAEYWNSQKDILEQARAADV
CRHNYGVVESFTVQR (SEQ ID NO: 121)

DR-2_{alpha} mini

IKEEHVILQAE**S**YLNPDQSGEF**K**FDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTK
RSNYTPI (SEQ ID NO: 122)

An example nucleic acid sequence encoding DRB1*1501 is provided below:

```
GGAGACACAAGACCCCGATTCTTGTGGCAGCCCCAAAAGGGAGTGCCATTTTTC AATGGGACGGAACGAGTTCGC
TTCCTTGATCGGTACTTTTACAACCAAGAAGAGAGTGTACGGTTCGACTCAGATGTCGGCGAGTTCGAGCGGTT
ACGGAATTGGGGCGACCTGACGCGGAGTACTGGAACCTCCAAAAGGATATTTTGGAGCAGGCACGAGCAGCTGTG
GACACCTATTGTCGACATAAATTATGGTGTGGTGGGAATCCTTTACAGTTCAGCGGCGGGTGCAACCTAAAGTGACC
GTGTATCCATCTAAAACGCAACCCCTCCAACACCATAACCTCCTGGTGTGTTCGTAAGCGGCTTCTATCCCGGG
TCAATTGAGGTCAGGTGGTTCCTCAACGGTCAGGAGGAGAAGGCCGGAATGGTAAGTACTGGTCTTATCCAGAAC
GGAGACTGGACCTTCCAAACTTTGGTAATGTTGGAAACGGTGCCGCGATCCGGGGAGGTGTATACATGCCAAGTT
GAACACCCGAGTGTACGAGCCCCCTGACG (SEQ ID NO: 402)
```

An example nucleic acid sequence encoding DRA*0101 is provided below:

```
ATAAAGAAGAGCACGTGATAATACAGGCGGAGTTTATTTGAACCCGGACCAGAGCGGTGAGTTCATGTTTCGAT
TTTGATGGCGACGAGATATTTACGTTGACATGGCAAAAAAGGAAACGGTGTGGAGACTTGAGGAGTTTGGACGA
TTCGCATCATTTGAGGCACAAGGAGCACTCGCCAATATCGCGGTGGACAAGGCCAACCTGGAGATCATGACAAAA
CGCTCCAATTATACGCTATCACTAATGTGCCCCCTGAGGTTACTGTGCTCACAAATTCTCCCGTAGAAGCTTAGG
GAACCTAACGTCCTCATATGTTTCATCGACAAGTTCACCTCCTCCGGTGGTCAATGTAACGTGGCTTCGGAATGGT
AAGCCGGTCACCACGGTGTCTCAGAGACCGTATTTCTGCCAGAGAGAAGACCACCTCTCCGCAAATTTCAATTAC
CTTCCCTTTCTTCTTCAACGGAAGACGTTTACGACTGCAGGGTCAACATTTGGGGGCTTGACGAGCCACTTCTC
AAGCATTGG (SEQ ID NO: 415)
```

Any of MHC class I and MHC class II constructs described herein can be further fused to one or more signaling domains and optionally one or more of the additional components. In some instances, the MHC-CAR constructs described herein are free of singling domains.

Preferably, a MHC-CAR as described herein contains matched MHC moiety and antigenic peptide, *e.g.*, a MHC molecule that would present the antigenic peptide or homologous analogs in natural state. In some instances, a MHC-CAR described herein may contain an alpha chain or a beta chain from HLA DRB1*1501 and an antigenic peptide associated with this HLA allele, *e.g.*, those MBP peptides described herein and others as well. The association between antigenic peptides involved in an autoimmune disease and a specific HLA allele is well known in the art or can be identified via routine practice, for example, library screening.

One exemplary MHC-CAR may have the following formula (+/- means that the specific component is optional):

Single chain (MHC Class I or II + peptide) (+/-hinge) + single chain CD28/4-1BB (+/-dileucine motifs) (+/-cd3zeta)). (Additional short peptide linkers can be added between components as described previously.)

Other exemplary MHC-CAR designs (single chain and multi-chain) are illustrated in Figure 10. In the case of multi-chain constructs, one or more short hinges may be used to enhance successful expression of the MHC-CAR. Further, it may be desirable to replace a

portion of the structure with conserved domains from mice domains to prevent cross-reactivity. Note that in some cases, the internal domain may only be attached to one of the chains.

The amino acid sequence of a MHC-CAR binding (that displays MBP) TCR is provided below:

TCR alpha MBP:

```
METLLGVSLVILWLQLARVNSQQGEEDPQALSIQEGENATMNC SYKTSINN LQWYRQNSGRGLVHLLILRS
NEREKHSGRLRVTLDTSKKSSLLITASRAADTASYFCATAAVGGFKTIFGAGTRLFVKANIQNPDPAVYQ
LRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKTVLDMRSMDFKSN SAVAWSNKSD FACANAFNNSII
PEDTFFPSPRESSCDVKLVEKSFETDTNLNFQNL SVIGFRILLKLVAGFNLLMTLRLWSS (SEQ ID NO:
123)
```

TCR beta MBP

```
MLLLLLLLGLAGSGLGAVVSQHPSWVISKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMATSNEGSKA
TYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYIC SARDLTSGANNEQFFGPGTRLTVLEDLKNVFPP
EVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQLKEQPALNDSRYSLSSRL
RVSATFWQNPRNHFRQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEI
LLGKATLYAVLVLSALVLMAMVKRKDSRG (SEQ ID NO: 124)
```

TCR alpha class I

```
mamlilgasvl ilwlqpdwvn sqqkndd
QQVKQNSPSSLVQEGRISILNCDYTNSMFDYFLWYKKYPAEGPTFLISISSIKDKNADGR
FTVFLNKS AKHLSLHIVPSQPGDSAVYFCAAMEGAQKL VFGQGTRLTINPNIQNPDPVAVY
QLRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKTVLDMRSMDFKSN SAVAWSNKSD
FACANAFNNSIIPEDTFFPSPRESS
Cdvklve ksfetdtlnl fqnlsvigfr illiklvagfn llmtlrlwss (SEQ ID NO: 125)
```

TCR beta class I:

```
msigllccaa lsliwagpv
NAGVTQTPKFQVLKTGQSM TLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVGAGITDQGEV
PNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYPGGGFYEQYFPGPTRLTVTEDLKNV
FPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQLKEQ
PALNDSRYALSSRLRVSATFWQDPRNHFRQVQFYGLSENDEWTQDRAKPVTQIVSAEAW
GRAD
cgftse sygggvlsat ilyeillgka tlyavlvsal vlmamvkrkd srg (SEQ ID NO: 126)
```

The amino acid sequences of exemplary CD19 targeting CAR constructs are provided below (note that these designs contain a 4-1BB domain which may be replaced with a cd28 domain):

4G7-CAR version 1:

MALPVTALLLLPLALLLHAARPEVQLQQSGPELIRPGASVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYINPY
NDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARGTYYYGSRVFDYWGQGTTLTVSSGGGGSGGG
GSGGGGSDIVMTQAAPSIPTVPGESVSISSRSKSLNSNGSGSGTAFTLRISRVEAEDVGVIYCMQHLEYFF
TAGTKLELKRSDPTTTPAPRPPTPAPTIASQFLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCKRGRKKLLYIFKQPFMRPVQTTEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNL
GRREYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMRGERRRGKGHDGLYQGLSTATKDTY
DALHMQALPPR (SEQ ID NO: 127)

4G7-CAR version 2:

METDTLLLLWV LLLWVPGSTG EVQLQQSGPE LIKPGASVKM SCKASGYTFT SYVMHWVKQK
PGQGLEWIGY INPYNDGTKY NEKFKGKATL TSDKSSSTAY MELSSLTSED SAVYYCARGT
YYYGSRVFDY WGQGTTLTVS SGGGGSGGGG SGGGGSDIVM TQAAPSIPTV PGESVSISSR
SSKSLNSNG NTYLYWFLQR PGQSPQLLIY RMSNLASGVP DRFSGSGSGT AFTLRISRVE
AEDVGVIYCM QHLEYFFTFG AGTKLELKRSDPTTTPAPRP PTPAPTIASQ PLSLRPEACR
PAAGGAVHTR GLDFACDIYI WAPLAGTCGV LLLSLVITLY CKRGRKKLLY IFKQPFMRPV
QTTEEDGCS CRFPEEEEGG CELRVKFSRS ADAPAYQQGQ NQLYNELNLG RREYDVLDK
RRGRDPEMGG KPRRKNPQEG LYNELQKDKM AEAYSEIGMK GERRRGKGHD GLYQGLSTAT
KDTYDALHMQ ALPPR (SEQ ID NO: 128)

The nucleic acid sequences of exemplary CD19 targeting CAR constructs are provided below.

4G7-CAR version 2:

atggagacagacactcttctcctttgggtcttctgctgctgtgggttcccggaagcacaggagaagtacagttgcaa
cagtctggggccagaactcatcaaaacccggagcttctgtaaaaatgtcatgcaaagctagtggatatacatttact
tcttaagtgatgcactgggtaaaaacagaaacctgggtcaggggcttgagtgatcggttacattaacccatataat
gacggcaccaaatataacagagaaattcaagggaaggctacgcttacatcagataagtccagtagcaccgcttat
atggaacttagcagccttacttcgaagattccgcggtgtattactgcgcgagagggaacttactactacgggagt
cgagttatcgattattgggggtcaaggcacgacgctcacggtgagctcaggtgggtggagggtctgggggtggcggc
agtgggtggggggggtcagacatcgtgatgacccaggcagcaccttctatcccggttaacccaggcgagctctgta
tctatcagttgtcgggtccagcaagctctcttctcaacagtaattggcaatacatatcttactgggttcctccaaagg
cctgggcaaaagtcctcaactcttcttatatcggatgtccaatcttgcgagtggtcggtaccccgacaggttttcagg
tctgggagcggaacagctttttacgttgagaatatccagggtagaagctgaggacgtcggtgtatattattgcatg
caacatctcgaataaccctttaccttcggcgctggtacaaagctcgaattgaaacgcagcgatccaaccacgacg
ccagcgccacgaccctacgcccgtctccaactattgcctcccagccccctgagttctcgccagaagcgtgtaga
cctgctgccggcggggcccgttcatacgcggggcttgactttgcatgtgatctatatatggggtcctttggcg
ggaaacttgcgagtgcttcttttgcactcgtgataacggttgattgtataaagggggtcgaaagaaactcctctat
atattttaagcagccctttatgaggcccggtgcaaacacacagaagaggacggatgctcttgcgattcccgga
gaggaggagggggggtgtgagcttaggggtcaagttttctcgtctcgcgacgcgcagcctatcaacagggccaa
aaccagctgtataacgaaactcaacctcgggcgcgggaagagtgatgacgtccttgacaaaaggcgcggtcgcgac
cctgaaatgggtggaaaaccgaggcgaaagaacccccaggagggaactttacaacgaattgcaaaaagacaagatg
gccgaagcctattccgaaattggaatgaaaggcgagcgagacgaggttaaggggcatgacggcctgtatcaaggg
ctctctacggccacgaaggatacttacgacgccccttcatatgcaagctcttccaccacgg (SEQ ID NO:
390)

MHC-CAR1 containing MHC-CAR1 part B (MHC-DRB CAR)- HLA DRB1*1501 (signal peptide), MBP peptide, HLA DRB1*1501 (external, hinge, transmembrane) CD3 ζ (cytoplasmic signaling domain) is provided below:

```
MVCLKLPGGSCMTALTFTLMVLSSPLALASDENPVVHFFKNIIVTPRTPPGGGGSGGGGSGGSGDTRPRFLWQPKR
ECHFFNGTERVRFLDRYFYNQEEVSFRFSDVGEFRAVTELGPRDAEYWNSQKDILEQARAADVITYCRHNYGVVES
FTVQRRVQPKVTVPYPSKTQPLQHHNLLVCSVSGFYPGSIEVRWFLNGQEEKAGMVSTGLIQNGDWTFTQTLVMLET
VPRSGEVYTCQVEHPSVTSPLTVEWRARSESAQSKMLSGVGGFVLGLLFLGAGLFIYFRNQTSRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDKRRGRDPENGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKRGHD
GLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO 412)
```

An example nucleic acid sequence encoding a MHC_CAR1 containing MHC-CAR1 part B (MHC-DRB CAR)- HLA DRB1*1501 (signal peptide), MBP peptide, HLA DRB1*1501 (external, hinge, transmembrane) CD3 ζ (cytoplasmic signaling domain) is provided below:

```
ATGGTATGCTTGAAGCTCCCGGGCGGGTCCTGCATGACCGCTCTCACTGTTACTCTTATGGTCCTTAGTTCACCG
CTTGCCCTGGCATCTGATGAGAATCCCGTGGTTCATTTTTTTAAGAACATCGTCACACCGCGCACCCACCTGGG
GGAGGCGGATCTGGCGGAGGCGGGAGTGGAGGCTCAGGAGACACAAGACCCCGATTCTTGTGGCAGCCCCAAAGG
GAGTGCCATTTTTTCAATGGGACGGAACGAGTTCGCTTCCCTTGATCGGTACTTTTACAACCAAGAAGAGAGTGTA
CGGTTTCGACTCAGATGTCGCGGAGTTCGAGCGGTTACGGAATTGGGGCGACCTGACGCGGAGTACTGGAACCTCC
CAAAAGGATATTTTTGGAGCAGGCACGAGCAGCTGTGGACACCTATTGTGACATAAATATGGTGTGGTGGAAATCC
TTTACAGTTTCAGCGCGGGTGCAACCTAAAGTGACCGTGTATCCATCTAAAACGCAACCCCTCCAACACCATAAC
CTCCTGGTGTGTTCGTAAGCGGCTTCTATCCCGGGTCAATTGAGGTGAGGTGGTTCCTCAACGGTCAGGAGGAG
AAGGCCGGAATGGTAAGTACTGGTCTTATCCAGAACGGAGACTGGACCTTCCAACTTTGGTAATGTTGGAAACG
GTGCCGCGATCCGGGGAGGTGTATACATGCCAAGTTGAACACCCGAGTGTACGAGCCCCCTGACGGTTGAGTGG
AGGGCGCGGTGAGAGAGCGCACAAATCTAAAATGCTGTGAGGAGTAGGCGGATTGTACTCGGACTCCTCTTTTTG
GGCGCTGGGTGTATTACTTTAGAAACCAACAAGTAGAGTAAAGTTTTCCCGAAGTGCGGACGCTCCCGCG
TATCAGCAAGGTCAAAACAGCTTTACAACGAACTGAACTTTGGACGACGCGAAGAGTACGATGTTCTTGATAAG
CGGAGAGGGCGCGATCCCGAAATGGGGGAAAGCCTCGGAGGAAGAACCACACAAGAAGGCCTTTATAATGAACTG
CAGAAGGACAAGATGGCGGAGGCGTATTCGAAATAGGCATGAAGGGTGAACGGAGGAGAGGAAAGGGACATGAC
GGACTTTATCAAGGATTGTCTACCGCAACTAAAGACACCTATGACGCGTTGCACATGCAGGCTCTCCCTCCGAGA
(SEQ ID NO: 413)
```

MHC-CAR containing MHC-CAR1 part A (MHC-DRA CAR) HLA-DRA*1010 (signal peptide, external, hinge, transmembrane), CD3 ζ (cytoplasmic signaling domain) is provided below:

```
MAISGVPVLGFFIIAVLMSAQESWAIKEEHVIIQAEFYLNPDQSGEFMFDFDGDGDEIFHV
DMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITNVPPEVTV
LTNSPVLELREPNVLICFIDKFTPPVVNVTLWRNGKPVTTGVSETVFLPREDHLFRKFH
YLPFLPSTEDVYDCRVEHWGLDEPLLKHWEFDAPSPLPETTENVVCALGLTVGLVGII
IGTIFIIKGLTSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEN
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKRGHDGLYQGLSTATKDT
YDALHMQALPPR (SEQ ID NO: 423)
```

An example nucleic acid sequence encoding a MHC-CAR1 part A (MHC-DRA CAR) HLA-DRA*1010 (signal peptide, external, hinge, transmembrane), CD3 ζ (cytoplasmic signaling domain) is provided below:

```
ATGGCAATATCTGGTGTTCCTGTCCTCGGGTTTTTTATCATAGCCGTAAGTGTGTCAGCACAGGAATCATGGGCG
ATAAAGAAGAGCACGTGATAATACAGGCGGAGTTTATTTGAACCCGGACCAGAGCGGTGAGTTCATGTTTCGAT
TTTGATGGCGACGAGATATTTACGTTGACATGGCAAAAAAGGAAACGGTGTGGAGACTTGAGGAGTTTGGACGA
TTCGCATCATTTGAGGCACAAGGAGCACTCGCCAATATCGCGGTGGACAAGGCCAACCTGGAGATCATGACAAAA
CGCTCCAATTATACGCTTATCACTAATGTGCCCCCTGAGGTTACTGTGCTCACAAATTCTCCCGTAGAACTTAGG
GAACCTAACGTCCTCATATGTTTCATCGACAAGTTCCTCCTCCGGTGGTCAATGTAACGTGGCTTCGGAATGGT
AAGCCGGTCACCACGGGTGTCTCAGAGACCGTATTTCTGCCAGAGAAGACCACCTCTTCCGCAAATTTCAATAC
CTTCCCTTTCTTCTTCAACGGAAGACGTTTACGACTGCAGGGTCGAACATTTGGGGGCTTGACGAGCCACTTCTC
AAGCATTGGGAGTTTCGACGCCCCATCACCGCTTCCAGAAACGACTGAAAACGTTGTCTGCGCTCTTGGCCTGACA
GTGGGCCTGGTAGGCATTATTATCGGGACCATCTTATCATCAAAGGTTTGACTTCCCGGTCAAATTTAGCAGA
TCCGCTGACGCACCGGCTACCAGCAGGGCCAGAACCACTCTACAACGAGCTGAATCTCGGCCGACGGGAAGAG
TATGACGTACTCGACAAGCGGAGAGGTGAGACCTGAGATGGGCGGTAAACCGAGACGGAAAAATCCCCAAGAG
GGTCTTTATAATGAACTCCAGAAGGATAAGATGGCTGAAGCCTATTCTGAGATAGGGATGAAAGGCGAGCGGCGG
AGGGGTAAGGGCCATGATGGCCTTTACCAGGGACTCTCCACGGCAACCAAAGATACTTACGACGCCCTTCACATG
CAAGCCCTCCCGCCACGC (SEQ ID NO: 424)
```

Construct 1 (CD19 CAR and CCR6 region) is provided below:

```
METDTLLLWVLLLWVPGSTGEVQLQQSGPELIKPGASVKMSCKASGYTFSTSYVMHWVKQKPGQGLEWIGYINPYN
DGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARGTYYYGSRVFDYWGQGTTLTVSSGGGGSGGGG
SGGGGSDIVMTQAAPSIPVTPGESVSIISRSSKSLNLSNGNTYLYWFLQRPQGSPQLLIYRMSNLASGVPDRFSG
SGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGAGTKLELKRSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE
EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQALPPRGSSSGSGEGRGSLLTCGDVEENPGPMSESM
NFSDVDFDSSEYFVSVNTSYYSVDSEMLLCSLQEVQRFSRLFPVPIAYSLLICVGLLGNILVVITFAFYKKARSMT
DVYLLNMAIADILFVLTLPFWAVSHATGAWVFSNATCKLLKGIYAINFNCGLLLTLCISMDRYIAIVQATKSFRL
RSRTLPRSKIIICLVVWGLSVIISSSTFVFNQKYNQGSQDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPLMFMI
FCYTFIVKTLVQAQNSKRHKAIRVIIAVVLVFLACQIPHNMVLLVTAANLGKMNRSQSEKLIGYTKTVTEVLAF
LHCCNLNPLYAFIQKFRNYFLKILKDLWCVRKRYKSSGFSAGRYSENI SRQTSETADNDNASSFTM (SEQ
ID NO: 425)
```

Construct 1 (CD19 CAR, CCR6, GFP region) is provided below:

```
METDTLLLWVLLLWVPGSTGEVQLQQSGPELIKPGASVKMSCKASGYTFSTSYVMHWVKQKPGQGLEWIGYINPYN
DGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARGTYYYGSRVFDYWGQGTTLTVSSGGGGSGGGG
SGGGGSDIVMTQAAPSIPVTPGESVSIISRSSKSLNLSNGNTYLYWFLQRPQGSPQLLIYRMSNLASGVPDRFSG
SGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGAGTKLELKRSDPTTTPAPRPPTPAPTIASQPLSLRPEACR
PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE
EEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM
AEAYSEIGMKGERRRRGKHDGLYQGLSTATKDTYDALHMQALPPRGSSSGSGEGRGSLLTCGDVEENPGPMSESM
NFSDVDFDSSEYFVSVNTSYYSVDSEMLLCSLQEVQRFSRLFPVPIAYSLLICVGLLGNILVVITFAFYKKARSMT
DVYLLNMAIADILFVLTLPFWAVSHATGAWVFSNATCKLLKGIYAINFNCGLLLTLCISMDRYIAIVQATKSFRL
RSRTLPRSKIIICLVVWGLSVIISSSTFVFNQKYNQGSQDVCEPKYQTVSEPIRWKLLMLGLELLFGFFIPLMFMI
FCYTFIVKTLVQAQNSKRHKAIRVIIAVVLVFLACQIPHNMVLLVTAANLGKMNRSQSEKLIGYTKTVTEVLAF
LHCCNLNPLYAFIQKFRNYFLKILKDLWCVRKRYKSSGFSAGRYSENI SRQTSETADNDNASSFTMGSGATNF
SLLKQAGDVEENPGPVSKGEELFTGVVPIILVELDGDVNGHKFSVSGEGEGDATYGKLTLLKFICTTGKLPVPWP
```

VTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELRGIDFKEDGN
ILGHKLEYNNYNSHNVYIMADKQKNGIKANFKIRHNIEDGSVQLADHYQONTPIGDGPVLLPDNHYLSTQSALS
PNEKRDMVLLLEFVTAAGITLGMDELYK (SEQ ID NO: 429)

An example nucleic acid sequence encoding Construct 1 (CD19 CAR, CCR6, GFP region) is provided below:

atggagacagacactcttctcctttgggtcttctgctgctgtgggttcccggaagcacaggagaagtacagttgcaa
cagtcctgggcccagaactcatcaaacccggagcttctgtaaaaatgtcatgcaaagctagtggatatacatttact
tcttacgtgatgcactgggtaaaaacagaaacctggtcaggggcttgagtggatcgggtacattaacccatataat
gacggccaccaaataaacagagaaattcaagggaagggtacgcttacatcagataagtcagtagcacccgttat
atggaacttagcagccttacttccgaagattccgcgggtgtattactgcgcgagagggacttactactacgggagt
cgagtattcgattattgggggtcaaggcacgacgctcacgggtgagctcaggtggtaggggtctgggggtggcggc
agtgggtgggggggggtcagacatcgtgatgaccaggcagcaccttctatcccggtaaccccgagtcagtcgtga
tctatcagttgtcgggtccagcaagtccttctcaacagtaattggcaatacatatctttactgggttcctccaaagg
cctgggcaagtcctcaacttcttatatatcggatgtccaatcttgcgagtggtgtaacccgacaggttttcaggg
tctgggagcgggaacagcttttacggttgagaatatccagggtagaagctgaggacgtcgggtgtatattattgcatg
caacatctcgaataacccctttaccttcggcgctgggtacaaagctcgaattgaaacgcagcgatccaaccacgacg
ccagcgccacgaccacctacgcccgcctccaactattgcctcccagcccctgagtcctcggccagaagcgtgtaga
cctgctgcccggcggggcccgttcatacgcggggccttgactttgcatgtgatctatatatggggtcctttggcg
ggaacttgccggagtgccttctttgtcactcgtgataacggttgattgtaaaaaggggtcgaaagaaactcctctat
atatttaagcagccctttatgagggccgtgcaaacacacagaagaggacggatgctcttgcgattcccgga
gaggaggaggggggtgtgagcttaggggtcaagttttctcgtctcgcgacgcgcccagccctatcaacagggccaa
aaccagctgtataacgaaactcaacctcggcgccgggaagagtatgacgtccttgacaaacggcgcggtcgcgac
cctgaaatgggtggaacccgagggcgaaagaaaccccgaggaggactttacaacgaattgcaaaaagacaagatg
gccgaagcctattccgaaattggaatgaaaggcgagcggagacgaggtgaagggcatgacggcctgtatcaaggg
ctctctacggccacgaaggatacttacgacgccttcatatgcaagctcttcaccacgggggttcgagcggcagt
ggagagggcgagaggaagtctgctaacatgcggtgacgtcgaggagaatcctggcccaatgagtggggaaagtatg
aacttcagcgatgtatttgactcctccgaagattactttgtatctgtgaatacagactattactccgtcgatagt
gaaatgctgctctgtagtctccaagaagtcggccaatccagtcgcctcttcgttcccatcgcgtactcccttatt
tgtgtttttggccttctgggtaacatcctggtgtaatcacattcgtttctataaaaaagctcggagtatgact
gatgtttaccttcttaacatggctatagcggacattcttttgtgcttactctccattctgggctgtgagccat
gcaacagggggtggttttttcaaagtccacatgtaagctgcttaaagggtatctatgcaataaaacttcaattgc
gggatgctcctgctgacatgcatcagtatggatcgatatacagctatagtagcagggcagtaagtccttcgcctg
cgatcccgcacactgcctaggagcaaaattatttgcctcgtcgtatgggggtctcagtgatcatctcctccagt
acgtttgtctttaaccagaaatataacacacaggggttctgatgtatgtgaaccaaagtatcagacagtgagtga
ccaatacgggtggaagttgcttatgttgggcttgagctgctttttgggtttttcatcccactgatgttcattgatt
ttctgtttatacatttattgttaagaccttggttcaggcgcaaaaatagcaagagacataaggcaattcgagtcac
attgcggtggtgttggctcttctggcctgtcagatccccacataatattggttctgctcgtcaccgcgctaaacttg
ggtaagatgaatcgatcttctcagtcggagaagttgatcggatacaccaaaactgtgacagaagtgctggccttc
cttactgttgtctgaacccagttttgtatgcttttataggacagaagtttcgaaattacttcttgaaaatcctc
aaggacctctggtgtgttcgaaggaagtacaagagctctggcctttagttgcgctggcgctacagtgagaatata
tcccggcagacctccgagactgctgataatgacaacgcaagttccttactatgggatccggcgcaacaaacttc
tctctgctgaaacaagccggagatgtcgaagagaatcctggaccgggtgagcaaggcgagggagctgttcaccggg
gtggtgcccactcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtctggcgagggcgagggc
gatgccacctacggcaagctgacctgaagttcatctgcaccacgggcaagctgccgctgcccgtggccaccctc
gtgaccacctgacctacggcgtgagtgcttcagcggctaccccgaccacatgaagcagcagcacttcttcaag
tccgccatgcccgaaggctacgtccaggagcgcaccatcttcttcaaggacgacggcgaactacaagacccgcgcc
gaggtgaagttcgagggcgacaacctggtgaaccgcatcgagctgaagggcatcgacttcaaggaggacggcaac
atcctggggcacaagctggagtacaactacaacgcccacaacgtctatatcatggccgacaagcagaagaacggc
atcaaggcgaaacttcaagatccggccacaacatcgaggacggcagcgtgcagctcggcgaccactaccagcagaac
acccccatcggcgacggcccccgtgctgctgcccgacaaccaactacctgagcaccagtcggccctgagcaagac

cccaacgagaagcgcgatcacatggctcctgctgagttcgtgaccgccgccgggatcactctcggcatggacgag
ctgtacaagtaa (SEQ ID NO: 430)

Construct 2 MHC CAR region (MHC-CAR1 part B, MHC-CAR1 part A region) is provided below:

MVCLKLPGGSCMTALTFTLMVLSSPLALASDENPVVHFFKNIVTPRTPPGGGGSGGGGSGGSGDTRPRFLWQPKR
ECHFFNGTERVRFLDRYFYNQEESVRFDSDVGEFRAVTELGPRDAEYWNQKIDILEQARAADVITYCRHNYGVVES
FTVQRRVQPKVTVPYSKTQPLQHHNLLVCSVSGFYPGSIEVRWFLNGQEEKAGMVSTGLIQNGDWTFTQTLVMLET
VPRSSEVYTCQVEHPSVTSPLTVEWRARSESAQSKMLSGVGGFVLGLLFLGAGLFYFRNQTSRVKFSRSADAPA
YQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDH
GLYQGLSTATKDTYDALHMQALPPRGSSGSGEGRGSLLTCCGVEENPGMAISGVPVLGFFIIAVLMSAQESWAI
KEEHVIIQAEFYLNPDQSGEFMFDFDGEI FHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKR
SNYTPITNVPPEVTVLTNSPVELREPVLICFIDKFTPPVVNVTLRNGKPVTTGVSETVFLPRDHLFRKFHYL
PFLPSTEDVYDCRVEHWGLDEPLLKHWEDAPSPLPETTENVVVCALGLTVGLVGLIIIGTIFIIKGLTSRVKFSRS
ADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR
GKGDHGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 431)

An example nucleic acid sequence encoding the Construct 2 MHC CAR region (MHC-CAR1 part B, MHC-CAR1 part A) is provided below:

atggtatgcttgaagctcccgggcggtcctgcatgaccgctctcactgttactcttatggtccttagttcaccg
cttgccctggcatctgatgagaatcccggtggttcatttttttaagaacatcgtcacacccggcaccacccctggg
ggaggcggatctggcggaggcgaggagtgaggctcaggagacacaaagaccccgattcttgtggcagcccaaaagg
gagtgcattttttcaatgggacggaacgagttcgtctccttgatcggtacttttacaaccaagaagagagtgt
cggttcgactcagatgtcggcgagttccgagcggttacggaattggggcgacctgacgggagtgactggaactcc
caaaaggatattttggagcaggcagcagcagctgtggacacctattgtcgacataattatggtgtggtggaatcc
tttacagttcagcggcggtgcaacctaaagtgaccgtgtatccatctaaaacgcaacccctccaacaccataac
ctcctggtgtgttccgtaagcggcttctatcccggtgcaattgaggtcaggtggttcccaacggtcaggaggag
aaggccggaatggttaagtactggtcttatccagaacgggagactggaccttccaaactttggtaatgttggaacg
gtgcccgcgatccggggagggtgtatacatgccaaagttgaacacccgagtggttacgagccccctgacggttgagtgg
aggcgcggtcagagagcgcacaaatctaaaatgctgtcaggagtaggcggattttgtaactcggaactcctcttttg
ggcgtggtgtgtttatctacttttagaaaccaaaacaaagtagagtaaagttttccgaagtgcggagcgtcccgcg
tatcagcaaggtcaaaaccagctttacaacgaaactggaactgggacgacgcgaagagtagcatgttcttgataag
cggagagggcgcgatcccgaaatggggggaaagcctcggaggaagaaccacacaagaaggcctttataatgaactg
cagaaggacaagatggcggaggcgtattccgaaataggcatgaagggtgaacggagagaggaaaggacatgac
ggactttatcaaggattgtctacccgcaactaaagacacctatgacgcgttgccatgcaggctctcctccgaga
ggttcgagcggcagtgagagggcgagaggaagtctgctaacatgcgggtgacgtcgaggagaatcctggcccaatg
gcaatatctggtgttctgtcctcgggttttttatcatagccgtactgatgtcagcacaggaatcatggcgata
aaagaagagcacgtgataatacaggcgagttttatgtgaacccggaccagagcggtgagttcatgttcgatttt
gatggcgacgagatatttcacgttgacatggcaaaaaaggaaacgggtgtggagacttgaggagtttgacgattc
gcatcatttgaggcacaaaggagcactcgccaatatcgcggtggacaaggccaacctggagatcatgacaaaacgc
tccaattatacgcctatcactaatgtgccccctgaggttaactgtgtcctacaaaattctcccgtagaacttagggaa
cctaacgtcctcataatgtttcatcgacaagttcactcctccgggtggtcaatgtaacgtggcttcggaatggttaag
ccggtcaccacgggtgtctcagagaccgtattttctgcccagagaagaccacctcttccgcaattttcattacctt
ccttttcttcttcaacggaagacgtttacgactgcagggtcgaacattgggggcttgacgagccacttctcaag
cattgggagttcgacgccccatcaccgcttcagaaaacgactgaaaacggtgtgtcgtctcttgccctgacagtg
ggcctggtaggcattattatcgggaccatctttatcatcaaagggttgacttcccggtcaaattagcagatcc
gctgacgcaccggcctaccagcaggggccagaaccaactctacaacgagctgaatctcggcgcagcgggaagagtat
gacgtactcgacaagcggagaggtcgagaccctgagatgggcggttaaaccgagacggaaaaatccccaagagggt
ctttataatgaactccagaaggataagatggctgaagcctattctgagatagggatgaaaggcgagcggcgagg

ggtaagggccatgatggcctttaccagggactctccacggcaaccaaagatacttacgacgcccttcacatgcaa
gcccctcccgccacgc (SEQ ID NO: 71)

Construct 2 kill switch and MHC CAR region (RQR8, MHC-CAR1 part B, MHC-CAR1 part A region) is provided below:

MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPSLCSGGGGG
PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRVC
KCPRPVVRSGSGQCTNYALLKLAGDVESNPGPPTGMVCLKLPGGSCMTALTVTLMVLSSPLALASDENPVVHFFK
NIVTPRTFPGGGGSGGGSGGSGDTRPRFLWQPKRECHFFNGTERVRFLDRYFYNQEESVRFSDVGEFRAVTEL
GRPDAEYWNSQKDI LEQARAADVITYCRHNYGVVESFTVQRRVQPKVTVYPSKTQPLQHHNLLVCSVSGFYPGSIE
VRWFLNGQEEKAGMVSTGLIQNGDWTFTQTLVMLETVPRSGEVYTCQVEHPSVTSPLTVEWRARSESAQSKMLSGV
GGFVLGLLFLGAGLFIYFRNQT SRVKFSRSADAPAYQQGQNQLYNELNLRREEYDVLDRRGRDPGEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSSGSGEGRGSLLTC
GDVEENPGPMAISGVPLGFFIIAVLMSAQESWAIKEEHVIIQAEFYLNPDQSGEFMFDFDGDDEIFHVDMAKKEP
VWRLEEFGRFASF EAQ GALANIAVDKANLEIMTKRSNYTPI TNVPPEVTVL TNSPVELREPNVLICFIDKFTFPV
VNVTLWRNGKPVTTGVSETVFLPREDHLFRKFHYLFLPSTEDVYDCRVEHWGLDEPLLKHWEFDAPSPLPETTE
NVVCALGLTVGLVGIIIGTIFI I KGLTSRVKFSRSADAPAYQQGQNQLYNELNLRREEYDVLDRRGRDPGEMGG
KPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID
NO: 211)

An example nucleic acid sequence encoding Construct 2 kill switch and MHC CAR region (RQR8, MHC-CAR1 part B, MHC-CAR1 part A region) is provided below:

atgggtacttcaactgttgctgctggatggcactttgtcttttgggtgccgatcatgctgatgcacgtccgtactcc
aatcctagcctgtgctccggggggggaggagtgaaactccctacacagggaaccttctctaattgtctccaccaac
gtctcccttgcaaaacccgaccacaacagcttgccctatagtaacccttccctctgtagtggaggggggggttca
cctgctccacgcctcctacccccgcgccaacgatcgcgctcacaaccgctcagctcttaggcgggaagcctgtagg
ccagcggctggcgggtgcgggttcatacgcggggattggattttgctgacatttacatttgggctccgctggcc
ggtaactgtggggattgtgctgttctctgttattacgctttattgcaatcacaggaaacaggcgacgagtatgc
aaatgcccgcggcccgctcgtagatctgggtccggccaatgtactaactacgctttgttgaaactcgctggcgat
gttgaaagtaaccccggtcctccaaacaggtatgggtatgcttgaagctcccgggcggtcctgcatgacgcctctc
actgttactcttatggtccttagttccacgcttgccctggcatctgatgagaatcccggtgggttcatttttttaag
aacatcgctcacaccgcgcacccccacctgggggaggcggatctggcgaggcgggagtgagggtcaggagacaca
agaccccgattcttgtggcagcccaaaaggagtgccattttttcaatgggacggaacgagttcgcttccttgat
cggtaacttttacaaccaagaagagagtgtagcgttcgactcagatgtcggcgagttccgagcggttacggaattg
ggcgacactgacgggagtagtactggaactcccaaaaggatattttggagcaggcagcagcagctgtggacacctat
tgtcgacataattatggtgtggtggaatcctttacagttcagcggcggtgcaacctaaagtgaacctgtatcca
tctaaaacgcaacccctccaaacaccataacctcctggtgtgttccgtaagcggtctctatcccggtcaattgag
gtcaggtggttccctcaacgggtcaggaggagaaggccggaatggtaagtagtactggtcttatccagaacggagactgg
accttccaaactttggtaatgttggaacgggtgcgcgcatccggggagggtgtatcatgccaagttgaacacccg
agtgttacgagccccctgacgggttagtgaggggcgcggtcagagagcgacacaatctaaaatgctgtcaggagta
ggcggattttgtactcggactcctctttttggcgctgggtgtttatctacttttagaaaccaaacaagtagagta
aagttttcccggaagtgcggacgctcccgctatcagcaagggtcaaaaccagctttacaacgaactgaacttggga
cgacgcgaagagtagatgttcttgataagcggagagggcgcgatcccgaaatgggggaaagcctcggagggaag
aaccacaagaaggcctttataatgaactgcagaaggacaagatggcgaggcggtattccgaaataggcatgaag
ggtgaacggaggagaggaaaggacatgacggactttatcaaggattgtctaccgcaactaaagacacctatgac
gcgttgacatgacggctctccctccgagagggttcgagcggcagtgaggaggcagagggaagtctgctaactatgc
ggtgacgtcgaggagaatcctggcccaatggcaatatctgggtgttctctgctcctcggttttttatcatagccgta
ctgatgtcagcacaggaatcatgggcgataaaagaagacgctgataatacaggcgagggtttttatttgaacccg

gaccagagcgggtgagttcatgttcgattttgatggcgacgagatattttcacgttgacatggcaaaaaaggaaacg
 gtgtggagacttgaggagtttggacgatttcgcatcattttgaggcacaaggagcactcgccaatatcgcggtggac
 aaggccaacctggagatcatgacaaaacgctccaattatacgccatcactaatgtgccccctgaggttactgtg
 ctcaaaaattctcccgtagaacttagggaacctaacgtccctcatatgtttcatcgacaagttcactcctccggtg
 gtcaatgtaacgtggcttcggaatggtaagccggtcaccacgggtgtctcagagaccgtattttctgccagagaa
 gaccacctcttcgcaaatctcattaccttcctttcttcttcaacggaagacgtttacgactgcagggtcgaa
 cattggggggttgacgagccacttctcaagcattgggagttcgacgccccatcaccgcttcagaaacgactgaa
 aacgttgctctgcgtcttggcctgacagtgggcttgtaggcattattatcgggaccatctttatcatcaaaggt
 ttgacttccgggtcaaatttagcagatccgctgacgcaccggcctaccagcagggccagaaccaactctacaac
 gagctgaatctcgccgacgggaagagtatgacgtactcgacaagcggagaggtcgagaccctgagatgggcggt
 aaaccgagacggaaaaatccccagagggtctttataatgaactccagaaggataagatggctgaagcctattct
 gagatagggtatgaaaggcgagcggcggagggttaagggccatgatggcctttaccagggaactctccacggcaacc
 aaagatacttacgacgcccccttcacatgcaagccctcccgccacgc (SEQ ID NO: 403)

Construct 2 (RQR8, MHC-CAR1 part B, MHC-CAR1 part A, GFP region) is provided below.

MGTSLLCWMALCLLGADHADACPYSNPCLSGGGGSELPTQGTFSNVSTNVSPAKPTTTACPYSNPCLSGGGGS
 PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRVC
 KCPRPVVRSGSQCTNYALLKLAGDVESNPGPTGMVCLKLPGGSCMTALTVTLMVLSSPLALASDENPVVHFFK
 NIVTPRTPPGGGSGGGGSGGSGDTRPRFLWQPKRECHFFNGTERVRFLLDRYFYNQESVRFDSVGEFRAVTEL
 GRPDAEYWNQKDI LEQARAADVITYCRHNYGVVESFTVQRRVQPKVTVPYPSKTQPLQHNNLLVCSVSGFYPGSIE
 VRWFLNGQEEKAGMVSTGLIQNGDWTFTLVMLETVPRSGEVYTCQVEHPSVTSPLTVEWRARSESAQSKMLSGV
 GGFVLGLLFLGAGLFIYFRNQT SRVKFSRSADAPAYQQGQNQLYNELNLGRREYDVLDKRRGRDPGEMGGKPRRK
 NPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSSGSSEGRGSLTLC
 GDVEENPGMAISGVVPLGFFII IAVLMSAQESWAIKEEHV I IQAIFYLNPDSGEFMFDFDGEI FHVDMAKKET
 VWRLEEFGRFASF EAQALANIAVDKANLEIMTKRSNYTPI TNVPPEVTVL TNSPVELREPNVLCIFIDKFTPPV
 VNVTLWRNGKPVTTGVSETVFLPREDHLFRKFHYLPFLPSTEDVYDCRVEHWGLDEPL LKHWEFDAPSPLPETTE
 NVVCALGLTVGLVGII IGTIFI I KGLTSRVKFSRSADAPAYQQGQNQLYNELNLGRREYDVLDKRRGRDPGEMGG
 KPRRKNPQEGLYNELQDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSGATNFSLL
 KQAGDVEENPGPVSKGEELFTGVVPILVELDGDVNGHKFSVSSEGEEDATYGKLT LKFICTTGKLPVPWP TLVTT
 LTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNKYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILG
 HKLEYNYSNHNVIYIMADKQKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLDNDHYLSTQSALSKDPNE
 KRDMVLLLEFVTAAGITLGMDELYK* (SEQ ID NO: 405)

An example nucleic acid sequence encoding Construct 2 (RQR8, MHC-CAR1 part B, MHC-CAR1 part A, GFP region) is provided below:

atgggtacttcaactgtgtgtggtggtggaacttctgtcttttgggtgccgatcatgctgatgcattgtccgtactcc
 aatcctagcctgtgctccggggggggaggagtgaaactccctacacagggaaccttctctaattgtctccaccaac
 gtctcccttgcaaaaccgaccacaacagcttgccctatagtaaccttccctctgtagtgagggggggggttca
 cctgtctccacgcctcctacccccgcgccaacgatcgcgctcacaaccgctcagtccttaggcgggaagcctgtagg
 ccagcgggtggcgggtgcggttcatacgcggggattggattttgcoctgcgacatttacatttgggtcccgctggcc
 ggtacttgtggggtattgtgtgtctcttattacgctttattgcaatcacaggaaacaggcgacgagtatgc
 aaatgcccgcggcccgctcgtagatctgggtccggccaatgtactaaactacgctttgttgaaactcgctggcgat
 gttgaaagtaaccccggtcctccaaacaggtatggatgcttgaagctcccgggcgggtcctgcatgaccgctctc
 actgttactcttatggtccttagttcaccgcttgccctggcatctgatgagaatcccggtgggttcatttttttaag
 aacatcgctcacaccgcgcacccccacctgggggaggcggatctggcgaggcgggagtgagggtcaggagacaca
 agaccccgattcttctggcagcccaaaaggagtgccattttttcaatgggacggaaacgagttcgcttccttgat
 cgggtactttttacaaccaagaagagagtgtacggttcgactcagatgtcggcgagttccgagcgggttacggaattg
 gggcgacctgacgcggagtagtctggaactcccaaaaggatatatttggagcaggcacgagcagctgtggacacctat

tgtcgacataattatggtgtggtggaatcctttacagttcagcggcgggtgcaacctaaagtgaacgtgtatcca
tctaaaacgcaacccctccaacaccataacctcctggtgtgttcctgtaagcggcttctatcccggtcaattgag
gtcaggtggttccctcaacggtcaggaggagaaggccggaatggtaagtaactggtcttattccagaacggagactgg
accttccaaactttggtaatgttggaaacggtgcccgcgatccggggagggtgtatcatgccaagttgaacacccg
agtgttacgagccccctgacggttgagtggaggggcgcggtcagagagcgcacaaatctaaaatgctgtcaggagta
ggcggatttgtactcggactcctctttttgggcgctgggtgtttatctacttttagaaaccaaacaagtagagta
aagttttcccgaaagtgcggacgctcccgcgatcagcaaggtcaaaaaccagctttacaacgaaactgaacttggga
cgaacggaagagtacgatgttcttgataagcggagagggcgcgatcccgaaatggggggaaagcctcggaggaa
aaccacagaagagcctttataatgaactgcagaaggacaagatggcggaggcgtattccgaaataggcatgaag
ggtgaacggaggagaggaaaggacatgacggactttatcaaggattgtctaccgcaactaaagacacctatgac
gcggtgcacatgcaggctctccctccgagagggttcgagcggcagtgagaggagggcagaggaagtctgctaactgc
ggtgacgtcaggagaaatcctggcccaatggcaatatctggtgttctctgctcgggttttttatcatagccgta
ctgatgtcagcacaggaatcatggcgataaaaagaagagcacgtgataatacaggcggagttttatttgaacccg
gaccagagcgggtgagttcatgttcgattttgatggcgacgagatatttcacgttgacatggcaaaaaaggaaacg
gtgtggagacttgaggagtttggacgattcgcattttgaggcacaaggagcactcgcgaatatcgcgggtggac
aaggccaacctggagatcatgacaaaacgctccaattatacgcctatcactaatgtgccccctgaggttactgtg
ctcacaatttctcccgtagaacttagggaacctaacgtcctcatatgtttcatcgacaagttcactcctccggtg
gtcaatgtaacgtggcttcggaatggtaagccggtcaccacgggtgtctcagagaccgtatttctgccagagaa
gaccacctcttccgcaatttctaccttcccttcttctccttcaacggaagacgtttacgactgcagggtcgaa
cattgggggcttgacgagccacttctcaagcattgggagtttcgacgccccatcaccgcttcagaaacgactgaa
aacgttgtctgctgcttctggcctgacagtggtgctgtaggcattattatcgggaccatctttatcatcaaaggt
ttgacttcccggtcaaattagcagatccgctgacgcacccggtcctaccagcagggccagaaccaactctacaac
gagctgaatctcggccgacgggaagagtatgacgtactcgacaagcggagaggtcgagacccctgagatggcggt
aaaccgagacggaaaaatccccaaagagggtctttataatgaactccagaaggataagatggctgaagcctattct
gagatagggatgaaaggcgagcggcggagggttaagggccatgatggcctttaccagggactctccacggcaacc
aaagatacttacgacgcttccatgcaagcctcctccgacacgggatccggcgcaacaaacttctctctgctg
aaacaagccggagatgtcgaagagaatcctggaccggtgagcaaggcgagaggctgttcacccgggtggtgcc
atcctggtcgagctggacggcgacgtaaacggccacaagttcagcgtgtctggcgaggggcgaggcgatgccacc
tacggcaagctgacctgaagttcatctgcaccacgggaagctgccgtgcccgtgcccggccacctcgtgaccacc
ctgacctacggcgtgacgtgttcagccgctaccccgaccacatgaagcagcagacttcttcaagtcggccatg
cccgaggctacgtccaggagcgcaccatcttcttcaaggacgacggcaactacaagaccggcgccgaggtgaag
ttcgaggggcgacacctggtgaaccgcatcgagctgaaggacatcgacttcaaggaggacggcaacatcctgggg
cacaagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggcatcaaggcg
aacttcaagatccgcccacaacatcgaggacggcagcgtgcagctcgccgaccactaccagcagaacacccccatc
ggcgacggccccgtgctgctgcccgaacaccactacctgagcaccagctccgcccgtgagcaaaagaccccaacgag
aagcgcgatcacatggtcctgctggagttcgtgaccgcccggggtcactctcgccatggacgagctgtacaag
taa` (SEQ ID NO: 409)

The amino acid sequences of exemplary CS-1 targeting CAR constructs are provided below
(note that these designs contain a 4-1BB domain which may be replaced with a CD28
domain):

Anti-CS1-CAR-v1 (Luc63-V1 CAR):

MALPVTALLLPLALLLHAARPEVKLLESGGGLVQPGGSLKLSAASGFDFSRYWMSWVRQAPGKGLEWIGEINPD
SSTINYTPSLKDKFII SRDNAKNTLYLQMSKVRSEDALYYCARPDGNYWYFDVWGAGTTVTVSSGGGSGGGGS
GGGGS DIVMTQSHKFMSTSVGDRVSITCKASQDVGIAVWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTD
FTLTISNVQSEDLADYFCQQYSSYPYTFGGGTGLEIKGLAVSTISSFFPPGYQKRGRKLLYIFKQPFMRPVQTT
QEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 129)

Anti-CS1-CAR-v2 (Luc63-V2 CAR):

MALPVTALLLPLALLLHAARPEVKLLESGGGLVQPGGSLKLSCAASGFDFSRYWMSWVRQAPGKGLEWIGEINPD
 SSTINYTPSLKDKFIISRDNANTLYLQMSKVRSEDTALYYCARPDGNYWYFDVWGAGTTVTVSSGGGGSGGGGS
 GGGGSDIVMTQSHKFMSTSVGDRVSITCKASQDVGIABAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTD
 FTLTISNVQSEDLADYFCQQYSSYPYTFGGGTKEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTR
 GLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
 KFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
 GERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 130)

Anti-CS1-CAR-v3 (Luc63-V3 CAR):

MALPVTALLLPLALLLHAARPEVKLLESGGGLVQPGGSLKLSCAASGFDFSRYWMSWVRQAPGKGLEWIGEINPD
 SSTINYTPSLKDKFIISRDNANTLYLQMSKVRSEDTALYYCARPDGNYWYFDVWGAGTTVTVSSGGGGSGGGGS
 GGGGSDIVMTQSHKFMSTSVGDRVSITCKASQDVGIABAWYQQKPGQSPKLLIYWASTRHTGVPDRFTGSGSGTD
 FTLTISNVQSEDLADYFCQQYSSYPYTFGGGTKEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIA
 RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
 APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF
 FLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPGKKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF
 PEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQEGLYNELQKDK
 MAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 131)

Anti-CS1-CAR-v4 (Luc90-V1 CAR):

MALPVTALLLPLALLLHAARPQVQLQPGGAELVRPGASVKLSCKASGYSTFTTYWMNWVKQRPQGQGLEWIGMIHPS
 DSETRLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCARSTMIATRAMDYWGQGTSTVTVSSGGGGSGGGG
 SGGGGSDIVMTQSQKSMSTSVGDRVSITCKASQDVITGVAWYQQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGT
 DFTFTISNVQAEDLAVYYCQGHYSTPLTFGAGTKLELKLGLAVSTISSFFPPGYQKRGRKKLLYIFKQPFMRPVQT
 TQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 132)

Anti-CS1-CAR-v5 (Luc90-V2 CAR):

MALPVTALLLPLALLLHAARPQVQLQPGGAELVRPGASVKLSCKASGYSTFTTYWMNWVKQRPQGQGLEWIGMIHPS
 DSETRLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCARSTMIATRAMDYWGQGTSTVTVSSGGGGSGGGG
 SGGGGSDIVMTQSQKSMSTSVGDRVSITCKASQDVITGVAWYQQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGT
 DFTFTISNVQAEDLAVYYCQGHYSTPLTFGAGTKLELKTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT
 RGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV
 VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
 KGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 133)

Anti-CS1-CAR-v6 (Luc90-V3 CAR):

MALPVTALLLPLALLLHAARPQVQLQQPGAELEVRPGASVKLSCKASGYSTFTTYWMNWVKQRPQGQLEWIGMIHPS
 DSETRLNQKFKDKATLTVDKSSSTAYMQLSSPTSEDSAVYYCARSTMIATRAMDYWGQGTSVTVSSGGGGSGGGG
 SGGGGSDIVMTQSQKSMSTSVGDRVSI TCKASQDVITGVAWYQQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGT
 DFTFTISNVQAE DLAVYYCQQHYSTPLTFGAGTKLELKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMI
 ARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPFVLDSDGS
 FFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR
 FPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQK
 DKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 134)

Anti-CA1-CAR-v7 (Luc34-V1 CAR):

MALPVTALLLPLALLLHAARPQVQLQQSGAELELRPGASVKLSCKASGYTFTSYWMQWVKQRPQGQLEWIGAIYPG
 DGDTRYTQKFKGKATLTADKSSSTAYMQLSSSLASEDSAVYYCARGKVYYSNPFAYWGQGT LVTVSAGGGGSGGG
 GSGGGGSDIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLIISGATSLETGVPSRFSGSGSG
 KDYTLSITSLQTEDVATYYCQQYWSTPWTFGGGTKEIKGLAVSTISSFFPGYQKGRKKLLYIFKQPFMRPVQ
 TTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKN
 PQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO:
 135)

Anti-CS1-CAR-v8 (Luc34-V2 CAR):

MALPVTALLLPLALLLHAARPQVQLQQSGAELELRPGASVKLSCKASGYTFTSYWMQWVKQRPQGQLEWIGAIYPG
 DGDTRYTQKFKGKATLTADKSSSTAYMQLSSSLASEDSAVYYCARGKVYYSNPFAYWGQGT LVTVSAGGGGSGGG
 GSGGGGSDIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLIISGATSLETGVPSRFSGSGSG
 KDYTLSITSLQTEDVATYYCQQYWSTPWTFGGGTKEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH
 TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIG
 MKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 136)

Anti-CS1-CAR-v9 (Luc34-V3 CAR):

MALPVTALLLPLALLLHAARPQVQLQQSGAELELRPGASVKLSCKASGYTFTSYWMQWVKQRPQGQLEWIGAIYPG
 DGDTRYTQKFKGKATLTADKSSSTAYMQLSSSLASEDSAVYYCARGKVYYSNPFAYWGQGT LVTVSAGGGGSGGG
 GSGGGGSDIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLIISGATSLETGVPSRFSGSGSG
 KDYTLSITSLQTEDVATYYCQQYWSTPWTFGGGTKEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLM
 IARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
 LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPFVLDSDG
 SFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKKRGRKKLLYIFKQPFMRPVQTTQEEDGCSC
 RFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQ
 KDKMAEAYSEIGMKGERRRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 137)

Anti-CS1-CAR-v10 (LucX1-V1 CAR)

MALPVTALLLPLALLLHAARPQVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWVKQRPQGQLEWIGRIYPG
 DGDTRYNGKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARSTMIATGAMDYWGQGTSTVTVSSGGGGSGGGG
 SGGGGSETTVTQSPASLSMAIGEKVTIRCITSTDIDDDMNWYQQKPGEPKLLISEGNTLRPGVPSRFSSSGYGT
 DFVFTIENMLSEADVADYYCLQSDNLPFTFGGGTKLEIKGLAVSTISSFFPPGYQKRGRKKLLYIFKQPFMRPVQT
 TQEEEDGCSCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNP
 QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 138)

Anti-CS1-CAR-v11 (LucX1-V2 CAR)

MALPVTALLLPLALLLHAARPQVQLQQSGAELARPGASVKLSCKASGYTFTSYWMQWVKQRPQGQLEWIGAIYPG
 DGDTRYTQKFKGKATLTADKSSSTAYMQLSSLASEDSAVYYCARGKVYYSNPFAYWGQGTSTVTVSSAGGGSGGGG
 GSGGGSDIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLLISGATSLRPGVPSRFSGSGSG
 KDYTLTSLQTEDVATYYCQQYWSTPWTFGGGTKLEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH
 TRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEEDGCSCRFPEEEEGGCEL
 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
 KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 139)

Anti-CS1-CAR-v12 (LucX1-V3 CAR):

MALPVTALLLPLALLLHAARPQVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWVKQRPQGQLEWIGRIYPG
 DGDTRYNGKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARSTMIATGAMDYWGQGTSTVTVSSGGGGSGGGG
 SGGGGSETTVTQSPASLSMAIGEKVTIRCITSTDIDDDMNWYQQKPGEPKLLISEGNTLRPGVPSRFSSSGYGT
 DFVFTIENMLSEADVADYYCLQSDNLPFTFGGGTKLEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMI
 ARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSKNKA
 LPAIEKTIKAKGQPREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS
 FFLYSLKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKKRGRKKLLYIFKQPFMRPVQTTQEEEDGCSCR
 FPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQEGLYNELQK
 DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 140)

Anti-CS1-CAR-v13 (LucX2-V1 CAR):

MALPVTALLLPLALLLHAARPQVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWVKQRPQGQLEWIGRIYPG
 DGDTRYNGKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARSTMIATGAMDYWGQGTSTVTVSSGGGGSGGGG
 SGGGSDIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLIYSASYRYTGVDPDRFTGSGSGTD
 ETFTISSVQAEDLAVYYCQQHYSTPPYTFGGGTKEIKGLAVSTISSFFPPGYQKRGRKKLLYIFKQPFMRPVQT
 TQEEEDGCCRFPEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQ
 EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 141)

Anti-CS1-CAR-v14 (LucX2-V2 CAR):

MALPVTALLLPLALLLHAARPQVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWVKQRPQGQLEWIGRIYPG
 DGDTRYNGKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARSTMIATGAMDYWGQGTSTVTVSSGGGGSGGGG
 SGGGSDIVMTQSHKFMSTSVGDRVSITCKASQDVSTAVAWYQQKPGQSPKLLIYSASYRYTGVDPDRFTGSGSGT
 DFTFTISSVQAEDLAVYYCQQHYSTPPYTFGGGTKEIKTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH
 TRGLDFADIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEEDGCSCRFPEEEEGGCEL
 RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
 KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 142)

Anti-CS1-CAR-v15 (LucX2-V3 CAR):

MALPVTALLLLPLALLLHAARPOVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWVKQRPQGGLIEWIGRIYPG
 DGDTKYNGKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARSTMIATGAMDYWGQGTSVTVSSGGGSGGGG
 SGGGSDIVMTQSHKFMSTSVGDRVSICTKASQDVSTAVAWYQQKPGQSPKLLIYSASYRYTGVDPDRFTGSGSGT
 DFTFTISSVQAEDLAVYYCQQHYSTPPYTFGGGKLEIKEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLM
 IARTPEVCCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
 PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPFVLDSGGS
 FFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGKKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR
 FPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRDPEMGGKPRRKNPQEGLYNELQK
 DKMAEAYSEIGMKGERRRRGKGDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 143)

(iii) Preparation of MHC-CARs

Any of the MHC-CAR constructs described herein can be prepared by a routine method, such as recombinant technology. Methods for preparing the chimeric receptors herein involve generation of a nucleic acid or a nucleic acid set that encodes or collectively encodes a MHC-CAR construct (including a single polypeptide or two subunits). In some embodiments, the nucleic acid also encodes a self-cleaving peptide (*e.g.*, P2A, T2A, or E2A peptide) between the coding sequences for the two subunits of a MHC-CAR, or between the coding sequence for a MHC-CAR and the coding sequence for other genes to be co-expressed with the MHC-CAR in a host cell (see discussions below).

Sequences of each of the components of the MHC-CARs may be obtained via routine technology, *e.g.*, PCR amplification from any one of a variety of sources known in the art. In some embodiments, sequences of one or more of the components of the MHC-CARs are obtained from a human cell. Alternatively, the sequences of one or more components of the MHC-CARs can be synthesized. Sequences of each of the components (*e.g.*, domains) can be joined directly or indirectly (*e.g.*, using a nucleic acid sequence encoding a peptide linker) to form a nucleic acid sequence encoding the MHC-CAR, using methods such as PCR amplification or ligation. Alternatively, the nucleic acid encoding the MHC-CAR may be synthesized. In some embodiments, the nucleic acid is DNA. In other embodiments, the nucleic acid is RNA.

Any of the MHC-CAR proteins, nucleic acid encoding such, and expression vectors carrying such nucleic acid, all of which are within the scope of the present disclosure, can be mixed with a pharmaceutically acceptable carrier to form a pharmaceutical composition, which is also within the scope of the present disclosure. "Acceptable" means that the carrier is compatible with the active ingredient of the composition (*e.g.*, the nucleic acids, vectors, cells, or therapeutic antibodies) and does not negatively affect the subject to which the

composition(s) are administered. Any of the pharmaceutical compositions to be used in the present methods can comprise pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formations or aqueous solutions.

Pharmaceutically acceptable carriers, including buffers, are well known in the art, and may comprise phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; amino acids; hydrophobic polymers; monosaccharides; disaccharides; and other carbohydrates; metal complexes; and/or non-ionic surfactants. See, *e.g.* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover.

II. Genetically Engineered Immune Cells Expressing MHC-CARs

(i) MHC-CAR-expressing immune cells

Immune cells expressing the MHC-CAR described herein provide a specific population of cells that can recognize pathogenic cells (*e.g.*, autoreactive T cells) involved in autoimmune diseases via MHC/peptide-TCR engagement. The interaction between the MHC-peptide portion of the MHC-CAR and the cognate TCR on the pathogenic cells would activate the MHC-CAR expressing immune cells via the signaling domains(s) of the MHC-CAR (optionally by recruiting cell membrane signaling molecules of the immune cells), leading to proliferation and/or effector functions of the MHC-CAR-expressing immune cells, which in turn eliminate the pathogenic cells. The immune cells can be T cells, NK cells, macrophages, neutrophils, eosinophils, or any combination thereof. In some embodiments, the immune cells are T cells. In some embodiments, the immune cells are NK cells. Specific examples are provided in Examples below.

The population of immune cells can be obtained from any source, such as peripheral blood mononuclear cells (PBMCs), bone marrow, tissues such as spleen, lymph node, thymus, tumor tissue, or established cell lines. A source suitable for obtaining the type of immune cells desired would be evident to one of skill in the art. In some embodiments, the population of immune cells is derived from PBMCs. The type of immune cells desired (*e.g.*, T cells, NK cells, macrophages, neutrophils, eosinophils, or any combination thereof) may be expanded within the population of cells obtained by co-incubating the cells with stimulatory molecules, for example, anti-CD3 and anti-CD28 antibodies may be used for expansion of T cells.

To construct the immune cells that express any of the MHC-CAR constructs described herein, expression vectors for stable or transient expression of the chimeric receptor construct may be constructed via conventional methods as described herein and introduced into immune host cells. For example, nucleic acids encoding the MHC-CAR may be cloned into a suitable expression vector, such as a viral vector (*e.g.*, a lentiviral vector) in operable linkage to a suitable promoter. The nucleic acids and the vector may be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of the nucleic acid encoding the chimeric receptors. The synthetic linkers may contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/plasmids/viral vectors would depend on the type of host cells for expression of the chimeric receptors, but should be suitable for integration and replication in eukaryotic cells.

A variety of promoters can be used for expression of the MHC-CAR constructs described herein, including, without limitation, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the Rous sarcoma virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, herpes simplex tk virus promoter. Additional promoters for expression of the chimeric receptors include any constitutively active promoter in an immune cell. Alternatively, any regulatable promoter may be used, such that its expression can be modulated within an immune cell.

Additionally, the vector may contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in host cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; T7 and SP6 RNA promoters for in vitro transcription of sense and antisense RNA; a “suicide switch” or “suicide gene” which when triggered causes cells carrying the vector to die (*e.g.*, HSV thymidine kinase, an inducible caspase such as iCasp9), and reporter gene for assessing expression of the MHC-CAR.

In some embodiments, the marker/sorting/suicide molecules for use in the present disclosure can be used for killing with rituximab and/or for sorting with QBEND. Philip et

al., Blood 124(8):1277-87; 2014). One example is RQR8, which contains rituximab mimotope and QBEND-10 epitope. Exemplary sequences are provided below:

MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSTNVSTNVSPAKPTTTACPYSNPSLC
SGGGGSPAPPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRN
RRRVCKCPRPVV (SEQ ID NO: 144)

MGTSLLCWMALCLLGADHADACPYSNPSLCSGGGGSELPTQGTFSTNVSTNVSPAKPTTTACPYSNPSLC
SGGGGSPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHR
NRRRVCKCPRPVV (SEQ ID NO: 394)

An exemplary nucleic acid sequence encoding a RQR8 is provided below.

ATGGGTACTTCACTGTTGTGCTGGATGGCACTTTGTCTTTTGGGTGCCGATCATGCTGATGCATGTCCGTACTCC
AATCCTAGCCTGTGCTCCGGGGGGGGAGGGAGTGAACCTCCCTACACAGGGAACCTTCTCTAATGTCTCCACCAAC
GTCTCCCCTGCAAAACCGACCACAACAGCTTGCCCCCTATAGTAACCTTCCCTCTGTAGTGGAGGGGGGGTTCA
CCTGCTCCACGCCCTCTACCCCCGCGCCAACGATCGCGTCACAACCGCTCAGTCTTAGGCCGGAAGCCTGTAGG
CCAGCGGCTGGCGGTGCGGTTTCATACGCGGGGATTTGATTTTGCCTGCGACATTTACATTTGGGCTCCGCTGGCC
GGTACTTGTGGGGTATTGCTGTTGTCTCTTGTATTACGCTTTATTGCAATCACAGGAACAGGCGACGAGTATGC
AAATGCCCGCGGCCCGTCTGTG (SEQ ID NO: 395)

In another example, the following exemplary RQR sequence tag can be affixed to a MHC-CAR construct as disclosed herein:

ACPYSNPSLCSGGGGSELPTQGTFSTNVSTNVSPAKPTTT**ACPYSNPSLCSGGGG** (SEQ ID NO: 145)

The boldfaced fragment is the rituximab minotope and the underlined/italicized fragment is the QBEND-10 epitope.

Suitable vectors and methods for producing vectors containing transgenes are well known and available in the art. Any of the vectors comprising a nucleic acid sequence that encodes a MHC-CAR construct described herein is also within the scope of the present disclosure. Such a vector may be delivered into host immune cells by a suitable method. Methods of delivering vectors to immune cells are well known in the art and may include DNA electroporation, RNA electroporation, transfection reagents such as liposomes, or viral transduction. In some embodiments, the vectors for expression of the MHC-CAR are delivered to host cells by viral transduction. Exemplary viral methods for delivery include, but are not limited to, recombinant retroviruses (see, *e.g.*, PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Pat. Nos. 5,219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent

No. 0 345 242), alphavirus-based vectors, and adeno-associated virus (AAV) vectors (see, *e.g.*, PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). In some embodiments, the vectors for expression of the chimeric receptors are retroviruses. In some embodiments, the vectors for expression of the chimeric receptors are lentiviruses.

In examples in which the vectors encoding chimeric receptors are introduced to the host cells using a viral vector, viral particles that are capable of infecting the immune cells and carry the vector may be produced by any method known in the art and can be found, for example in PCT Application No. WO 1991/002805A2, WO 1998/009271 A1, and U.S. Patent 6,194,191. The viral particles are harvested from the cell culture supernatant and may be isolated and/or purified prior to contacting the viral particles with the immune cells.

Following introduction into the host cells a vector encoding any of the MHC-CAR provided herein, the cells are cultured under conditions that allow for expression of the chimeric receptor. In examples in which the nucleic acid encoding the MHC-CAR is regulated by a regulatable promoter, the host cells are cultured in conditions wherein the regulatable promoter is activated. In some embodiments, the promoter is an inducible promoter and the immune cells are cultured in the presence of the inducing molecule or in conditions in which the inducing molecule is produced. Determining whether the MHC-CAR is expressed will be evident to one of skill in the art and may be assessed by any known method, for example, detection of the chimeric receptor-encoding mRNA by quantitative reverse transcriptase PCR (qRT-PCR) or detection of the chimeric receptor protein by methods including Western blotting, fluorescence microscopy, and flow cytometry. See also Examples below. Alternatively, expression of the MHC-CAR may take place *in vivo* after the immune cells are administered to a subject.

Alternatively, expression of a MHC-CAR construct in any of the immune cells disclosed herein can be achieved by introducing RNA molecules encoding the MHC-CAR constructs. Such RNA molecules can be prepared by *in vitro* transcription or by chemical synthesis. The RNA molecules can then introduced into suitable host cells such as immune cells (*e.g.*, T cells, NK cells, macrophages, neutrophils, eosinophils, or any combination thereof) by, *e.g.*, electroporation. For example, RNA molecules can be synthesized and introduced into host immune cells following the methods described in Rabinovich et al., Human Gene Therapy, 17:1027-1035 and WO WO2013/040557.

The methods of preparing host immune cells expressing any of the MHC-CARs described herein may comprise expanding the host immune cells *ex vivo*. Expanding host immune cells may involve any method that results in an increase in the number of cells expressing MHC-CAR, for example, allowing the host cells to proliferate or stimulating the host cells to proliferate. Methods for stimulating expansion of host cells will depend on the type of host cell used for expression of the chimeric receptors and will be evident to one of skill in the art. In some embodiments, the host immune cells expressing any of the MHC-CAR described herein can be expanded *ex vivo* prior to administration to a subject.

(ii) *Additional Genetic Modifications*

One or more additional genetic modifications can be introduced into host immune cells before, concurrently with, or after the transfection of the MHC-CAR construction. For example, one or more marker and/or suicide genes may be introduced into the host immune cells. Examples include green fluorescent protein (GFP), enhanced blue fluorescent protein (eBFP), and RQR genes, such as RQR8 (a compact marker/suicide gene for T cells which combines target epitopes from CD34 and CD20. Philip et al., Blood 124(8):1277-87; 2014). Such marker/suicide genes may be constructed in one expression cassette with the MHC-CAR components.

An example of an amino acid sequence of GFP is provided below:

VSKGEELFTGVVPI LVELDGDVNGHKFSVSGEGEGDATYGKLT LKFICTTGKLPVPWPTLVTTLT YGVQCFSRYP
DHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNV
YIMADKQKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPNDHYLSTQSALS KDPNEKRDMVLEFVT
AAGITLGMDELYK (SEQ ID NO: 427)

An example of a nucleic acid sequence encoding GFP is provided below:

gtgagcaaggggcgaggagctgttcacccgggtggtgccatcctcgtcgagctggacggcgacgtaaacggccac
aagttcagcgtgtctggcgagggcgagggcgatgccacctacggcaagctgacctgaagttcatctgcaccacc
ggcaagctgcccgtgccctggcccaccctcgtgaccacctgacctacggcgtgcagtgcttcagccgctacccc
gaccacatgaagcagcagcacttcttcaagtcggccatgcccggaaggctacgtccaggagcgcaccatcttcttc
aaggacgacggcaactacaagacccgcgcgaggtgaagttcgagggcgacacctggtgaaccgcacatcgagctg
aagggcacatcgacttcaaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaacgctc
tatatcatggccgacaagcagaagaacggcatcaaggcgaaacttcaagatccgccacaacatcgaggacggcagc
gtgcagctcggcaccactaccagcagaacacccccatcggcgacggccccgtgctgctgccgcacaaccactac
ctgagcaccagtcctgcctgagcaaacccccaacgagaagcggcgcacatggtcctgctggagttcgtgacc
gcgcgcgggatcactctcggcatggacgagctgtacaagtaa (SEQ ID NO: 428)

In some instances, the endogenous TCR (alpha chain, beta chain, or both) can be disrupted such that the host immune cells do not express the endogenous TCR. Deficiency in endogenous TCR could avoid undesired T cell activation. Alternative or in addition, certain cell surface receptors can be knocked out. Such surface receptors may be target receptors for

disease treatment, for example, CD52, which is a target for MS treatment. Knock-out such target receptors from the MHC-CAR immune cells allows for the co-use of the MHC-CAR immune cells with therapeutic agents specific to the target receptor (*e.g.*, and anti-CD52 antibodies such as alemtuzumab).

In some embodiments, the host immune cells may be modified with synthetic surface proteins to enhance their retention in a specific organ or tissue, for example, in the lymph nodes, in tertiary lymphoid organs, or at sites of inflammation. Doing so would allow the modified immune cells to access target pathogenic cells, while minimizing fatal off-target effects due to penetration of the blood brain barrier or free travel of the immune cells through peripheral blood. Cells early in the T cell differentiation pathway (*e.g.*, naïve, stem cell memory, and central memory T cells) travel freely to the lymph nodes. As differentiation progresses, most effector T cells leave the lymph node. Pathologic immune cells can also travel to and accumulate at sites of inflammation. Treatment by activated CAR-T cells has a number of undesirable effects when they react with undesired targets. Interaction with heart tissue can be fatal to cardiac protein, and permeation of the brain can lead to fatal cerebral edema. Recent progress has been made in treatment of the brain cancer glioblastoma using lower doses of CAR T therapy than in systemic treatments for cancer using CD19 CAR T therapy. Brown et al., *New England Journal of Medicine*, 375(26):2561-2569, 2016. Resolution of inflammation has the potential to transform pathologic to protective environments. Gagliani et al., *Nature*, 523(7559):221-225, 2015.

Introducing one or more of lymph node retention proteins into the immune cells can enhance retention of the immune cells in the lymph node, wherein the immune cells still have access to target pathogenic cells, while undesired effects as noted above can be significantly reduced. Naïve lymphocytes enter the lymph node via high endothelial venules (HEVs). Thus, expressing or overexpressing proteins involved in HEV anchoring and/or entry can facilitate the immune cells entering into lymph node. Exemplary lymph node retention proteins include, but are not limited to, CCR7 (a chemokine receptor), MECA79 (a peripheral lymph node addressin), vascular adhesion protein-1 (VAP-1) and CD62 (selectin, a family of the cell adhesion molecules). Azzi et al., *Blood* 124(4):476-477, 2016; Streeter et al., *J. Cell. Biol.* 107:1853-186; 1988; Michie et al., *Amer. J. Path.* 143:1688-1698; 1993; Berg et al., *J. Cell. Biol.* 114:343-349; 1991; Berg et al., *Nature* 366:695-698; 1993; and Hemmerich et al., *J. Exp. Med.* 180:2219-2226; 1994. Alternatively, genes encoding proteins (*e.g.*, sphingosine-1-phosphate receptor-1 or S1P) involved in lymphocyte egress from the thymus

and lymph organs can be knocked out from the immune cells.

Chemokine receptors and adhesion receptors that promote trafficking to sites of inflammation can also bring MHC-CAR immune cells in contact with pathogenic cells that propagate immune disease [Barreiro et al., *Cardiovascular research*, 86(2):174-182, 2010] see Table 3 and 4. Receptors involved in recruiting immune cells that propagate inflammation include receptors (*i.e.*, CXCR5, CCR7, CCR6) that recruit to tertiary lymphoid organs (where CXCL13, CCL19, CCL20, CCL21 are expressed).

One or more genes encoding proteins involved in targeting other organs/tissues, for example, brain/CNS, bone marrow, pancreas, intestine, liver, lungs, spleen, and/or thymus, may also be introduced into or knocked-out from the immune cells.

The genes (in Table 3 and 4), by means of virally induced or temporary RNA mediated expression (possibly combined with knockout of the endogenous gene) in the therapeutic cell, may be used to route either Treg or CTL cells to the desired location or to treat/remove the desired cells. Barreiro, et al. *Cardiovascular research*, 86(2):174-182, 2010. If mRNA transfection is utilized then it can allow expression of the chemokine or adhesion receptor for a week. Wang and Rivière. *Molecular Therapy-Oncolytics*, 3:16015 2016.

Table 3. Chemokine receptors and natural context

Receptor	Typically expressed on	GenBank Accession number	Ligands
CXCR1	Neutrophils	AAY21515.1	CXCL8, CXCL6
CXCR2	Neutrophils	NP_001548.1	CXCL1, 2, 3, 4, 5, 7
CXCR3	T cells and some B cells	NP_001495.1	CXCL9, 10, 11
CXCR4	Most mature and immature hematopoietic cells	CAA12166.1	CXCL12
CXCR5	B cells and Tfh cells	NP_001707.1	CXCL13
CXCR6	Inflammation response T cells but weak chemotaxis	NP_006555.1	CXCL16
CXCR7	Memory B cells, T cells	NP_064707.1	CXCL12

CCR1	Peripheral lymphocytes, memory T cells	NP_001286.1	CCL4, CCL5, CCL6, CCL14, CCL15, CCL16, CCL23
CCR2	Monocytes, activated memory T cells, B cells, basophils	AAA19119.1	CCL11, CCL26, CCL7, CCL13, CCL15, CCL24 & CCL5, CCL28
CCR3	Plays a role in allergic reactions, B and T cells to mucous, eosinophils, basophils	NP_001828.1	CCL11, CCL26, CCL7, CCL13, CCL15, CCL24, CCL5
CCR4	Th2 lymphocytes, dendritic cells	NP_005499.1	CCL3, CCL5, CCL17, CCL22
CCR5	Peripheral blood dendritic cells, CD34+ hematopoietic progenitors, activated/memory Th1	NP_000570.1	CCL2, CCL3, CCL4, CCL5, CCL11, CCL13, CCL14, CCL16
CCR6	Inactivated memory T cells, dendritic cells, Th17, downregulated on activated T cells	AAC51124.1	CCL20
CCR7	Trafficking of B, T, and dendritic cells across HEV and into T cell zone of lymph nodes	AAH35343.1	CCL19, CCL21
CCR8	Th2 cells, thymus, lymph nodes, spleen, brain, monocytes	NP_005192.1	CCL1, CCL16
CCR9	Thymus, gut	NP_112477.1	CCL25
CCR10	Skin, mucous layers, regulatory T cells	NP_057686.2	CCL27, CCL28
XCR1		NP_005274.1	XCL1, XCL2
CX3CR1		NP_001164642.1	CX3CL1

The amino acid sequence of CCR6, provided by AAC51124.1 is shown below:

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MSGESMNFSDVFDSSSEDFVSVNTSYYSVDSEMLLCSLQEVRFQSRFLFVPIAYSLLICVFGLLGNIILVVITFAFYK
KARSMTDVYLLNMAIADILFVLTLFFWAVSHATGAWVFSNATCKLLKGIYAINFNCGMLLLTCISMTRYIAIVQA
TKSFRLRSRTLPRSKIICLVVWGLSVLISSSTFVENQKYNTQGSVDVCEPKYQTVSEPIRWKLLMLGLELLFGFFI
PLMFMIFCYTFIVKTLVQAQNSKRHKAIRVIIAVVLVFLACQIPHNMVLLVTAANLGKMNRSQCSEKLIGYTKTV
TEVLAFLHCLNPNVLYAFIGQKFRNYFLKILKDLWCVRKYKSSGFSCAGRYSENISRQTSSETADNDNASSETM
(SEQ ID NO: 391)
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Example nucleic acid sequences of CCR6 are shown below:

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atgagtggggaaagtatgaacttcagcgatgtatttgactcctccgaagattactttgtatctgtgaatacagagc
tattactccgtcgatagtgaaatgctgctctgtagtcctccaagaagtcgccaattcagtcgctcttcgttccc
atcgcgtaactccttatttgggtgttttggcctctctgggtaacatcctggttgtaatacattcgtcttctataaa
aaagctcggagtatgactgatgtttaccttcttaacatggctatagcggacattcttttgggtgcttactctccca
ttctgggctgtgagccatgcaacaggggctgtgggttttttcaaagtcacatgtaagctgcttaaagggatctat
gcaataaaacttcaattgcgggatgctcctgctgacatgcacatcagtatggatcgatcacatagctatagtagcaggcg
actaagtccttccgctgctgcatcccgacactgcctaggagcaaaaattatttgcctcgtcgtatgggggctctca
gtgatcatctcctccagtagctttgtctttaaccagaaatataacacacaggggtctgatgtatgtgaaccaag
tatcagacagtgagtgaaaccaatacgggtggaagttgcttatgttgggcttgagctgcttttgggtttttcatc
ccactgatgttcatgattttctgttatacatttatgtttaagaccttggttcaggcgcaaaaatagcaagagacat
aaggcaattcgagtcacatcattgcggtggtgttgggtcttcttggcctgtcagatccccataatatggttctgtc
gtcaccgcccgttaacttgggtaagatgaatcgatcttgtcagtcgagaagttgatcggtacacacaaaactgtg
acagaagtgctggccttccctcactgttgtctgaacccagtttggatgtctttataggacagaagtttcaaat
tacttcttgaaaatcctcaaggacctctggtgtgttgaaggaagtacaagagctctggctttagttgcgctggg
cgctacagtgagaatatatcccggcagacctccgagactgctgataatgacaacgcaagttccttccactatg
(SEQ ID NO: 392)
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ATGAGCGGGGAATCAATGAATTTTCAGCGATGTTTTCGACTCCAGTGAAGATTATTTTGTG
TCAGTCAATACTTCATATTACTCAGTTGATTCTGAGATGTTACTGTGCTCCTTGCAGGAG
GTCAGGCGATTCTCCAGGCTATTTGTACCGATTGCCTACTCCTTGATCTGTGTCTTTGGC
CTCCTGGGGAATATTTCTGGTGGTGATCACCTTTGCTTTTATAAGAAGGCCAGGCTATG
ACAGACGTCTATCTCTTGAACATGGCCATTGCAGACATCCTCTTTGTTCTTACTCTCCCA
TTCTGGGCAGTGAGTCATGCCACTGGTGCGTGGGTTTTCAGCAATGCCACGTGCAAGTTG
CTAAAAGGCATCTATGCCATCAACTTTAACTGCGGGATGCTGCTCCTGACTTGCAATTAGC
ATGGACCGGTACATCGCCATTGTACAGGCGACTAAGTCATTCCGGCTCCGATCCAGAACA
CTACCGCGCACGAAAATCATCTGCCTTGTGTGTGGGGGCTGTGAGTCATCATCTCCAGC
TCAACTTTTGTCTTCAACCAAAAATACAACACCCAAGGCAGCGATGTCTGTGAACCCAAG
TACCAGACTGTCTCGGAGCCCATCAGGTGGAAGCTGCTGATGTTGGGGCTTGAGCTACTC
TTTGGTTTCTTTATCCCTTTGATGTTTATGATATTTTGTACACGTTTATTGTCAAAACC
TTGGTGCAAGCTCAGAATTCTAAAAGGCACAAAGCCATCCGTGTAATCATAGCTGTGGTG
CTTGTGTTTCTGGCTTGTGAGATTCTCATAACATGGTCTCTGCTTGTGACGGCTGCAAAAT
TTGGGTAAAATGAACCGATCCTGCCAGAGCGAAAAGCTAATTGGCTATACGAAAATGTC
ACAGAAGTCTTGGCTTCTTGCCTGCTGCTGCTGAACCTGTGCTTACGCTTTTATTTGGG
CAGAAGTTCAGAACTACTTTCTGAAGATCTTGAAGGACCTGTGGTGTGTGAGAAGGAAG
TACAAGTCTCAGGCTTCTCCTGTGCGGGAGGTACTCAGAAAACATTTCTCGGCAGACC
AGTGAGACCGCAGATAACGACAATGCGTCTCTTCACTATG (SEQ ID NO: 393)
```

Table 4. Adhesion receptors and natural context

Receptor	Typically expressed on	Accession number	Ligands
VLA-1 or $\alpha_1\beta_1$	Many cell types	NP_852478.1, AAH20057.1	Collagens, laminins
VLA-2 or $\alpha_2\beta_1$	Many cell types	NP_002194.2, AAH20057.1	Collagens, laminins
VLA-3 or $\alpha_3\beta_1$	Many cell types	AAI50191.1, AAH20057.1	Laminin-5
VLA-4 or $\alpha_4\beta_1$	Hematopoietic cells	NP_000876.3, AAH20057.1	Fibronectin and proteinases
VLA-5 or $\alpha_5\beta_1$	Many cell types	NP_002196.4, AAH20057.1	Fibronectin, VCAM-1
VLA-6 or $\alpha_6\beta_1$	Many cell types	AAI36456.1, AAH20057.1	Laminins
$\alpha_4\beta_7$	Gut	NP_000876.3, NP_000880.1	MADCAM1
$\alpha_7\beta_1$	Muscle	AAQ89241.1, AAH20057.1	Laminins
$\alpha_L\beta_2$	T lymphocytes	NP_002200.2, NP_000202.3	I-CAM1, I-CAM2
MAC-1 or $\alpha_M\beta_2$	Neutrophils and monocytes	AAB24821.1, NP_000202.3	I-CAM1
$\alpha_{IIb}\beta_3$	Platelets	AAI26443.1, AAI27668.1	Fibrinogen, fibronectin
$\alpha_v\beta_1$	Melanocytes	AAA61631.1, AAH20057.1	Vitronectin, fibrinogen
$\alpha_v\beta_3$	Activated endothelial cells	AAA61631.1, AAI27668.1	Vitronectin, fibronectin, fibrinogen, osteopontin, Cyr61, tyroxine, Tetrac
$\alpha_v\beta_5$	Epithelial cells and fibroblasts	AAA61631.1, NP_002204.2	Vitronectin

$\alpha_V\beta_6$	Lung, mammary gland	AAA61631.1, NP_000879.2	Fibrinonectin and TGF- β 1, 3
$\alpha_V\beta_8$	Neural tissues	AAA61631.1, NP_002205.1	Fibrinonectin and TGF- β 1, 3
$\alpha_6\beta_4$	Epithelial cells	AAA61631.1, CAB61345.1	Laminin

Alternatively or in addition, genes that may enhance immune cell functions, *e.g.*, proliferation, cytotoxicity, etc., can also be introduced into or knocked-out from the immune cells. Examples include TNF/TNFR2 overexpression (for short-lived but more effective CD8 T cells), *gld* (FasL mutant; for lymphoproliferation; CTLs do not kill via Fas-FasL pathway); *lpr* (Fas mutant; for upregulation of FasL – target cells resistant to FasL-mediated apoptosis); Granzyme B* deficient (delayed nuclear apoptotic changes in target cells); Granzyme A & B* deficient (delayed nuclear apoptotic changes in target cells); Perforin deficient (complete absence of granule-mediated apoptosis); Perforin and FasL deficient (defective granule-mediated and Fas-mediated apoptosis); Cathepsin C (dipeptidyl-peptidase I) deficient (fails to produce active granzymes and some haematopoietic serine proteases); FAS (CD95) underexpression; and/or FASL overexpression.

Table 5 below lists additional genetic modifications of the MHC-CAR T cells or co-treatment described herein and the accompanying advantages arising therefrom.

Table 5. Genetic Modifications or Co-Treatment and Benefits Thereof

Genetic Modifications	Advantages
- TCR knockout - CIITA deletion (to remove endogenous MHC class II expression) - CS-1 (CD319) deletion (which is present on T cells)	Reduce fraternal killing of MHC-CAR T cells by natural CD8 ⁺ T cells with an affinity to the peptide-MHC in the MHC-CAR, thereby extending the life-span of the MHC-CAR T cells. (Without the genetic modifications, the MHC-CAR T cells would still be cytotoxic and effective, but would have a short life-span.)
- Fas deletion, which optionally can be in combination of FasL overexpression - PD-1 deletion, which optionally can be in combination with PD-L1 and/or PD-L2 overexpression	Enhance activity to eliminate CD8 cytotoxic T cells, antigen-presenting cells (APCs), and/or B cells

- Co-express of CS-1 CAR (conventional CAR construct having an extracellular domain specific to CS-1) - Co-express of CD19 CAR (conventional CAR construct having an extracellular domain specific to CD19)	
- PD-L1 overexpression, which may optionally be in combination with PD-1 deletion - PD-L1 + CTLA4-Ig, which may optionally may be in combination with PD-1 deletion - FasL overexpression, which may optionally be in combination with Fas deletion - Galectin 9 expression, which may optionally be in combination with Tim-3 deletion - CS-1 deletion (when CS-1 CAR is used to reduce fraternal killing) - Include a hinge in a MHC-CAR	Reduce the level of MHC-CAR T cell elimination by other immune cells; Inclusion of hinge may decrease/prevent killing with cell TCR by, e.g., decreasing ability for it to engage CD4 or CD8
- TCR knockout when allogenic cells are used (cells can be sorted to remove non-edited cells)	Reduce the risk of graft-v-host disease
- Treg cells expressing MHC-CAR - PD-1 and/or PD-L1 knockout - CXCR5 expression	Reduce humoral responses to the peptide-MHC of interest (e.g., antibody responses), especially a B cell response
- IL-35 expression - Inhibitors (e.g., antibodies) targeting cytokine producing B cells (e.g., targeting CD10) and/or other activated immune cells (e.g., targeting CS-1)	Reduce inflammation and/or enhance tolerogenic environment
-Relevant chemokine receptor expression to either direct to relevant organ (Schall et al., Nature Reviews Immunology, 11(5):355-363, 2011) or to interact with relevant cell type (example, CXCR5 for B cell, CCR6 for Th17) -Antigen targeting antibody scFv that contains a CD8 hinge, a transmembrane domain, and optional stimulatory and co-stimulatory domain (for Treg only). (for example an anti-MOG antibody). The relevant antibody sequence (for a subset of antigen targets in Table 1) can be	Routing to inflamed or antigen presenting or antigen targeting environment

generated from sequencing of commercially available human-targeted monoclonal variants using mass spectrometry. Tran et al. Scientific reports, 6:31730, 2016.	
- Genetically encoded kill-switches	Reduce cytokine crisis

In some embodiments, genetic modification that lead to PD blockade can be introduced into the immune cells that express a MHC-CAR as described herein. Such modifications include one or more of PD-1 knockout, PD-L1 or L2 overexpression, or PD-L1 knockout. PD blockade may be combined with an immune-inhibitor (*e.g.*, knockouts of CTLA-4, TIM-3, LAG3, TIGIT, IDO, or Arginase, or CTLA-4lg secretion), an immunostimulator (*e.g.*, anti-OX40, anti-CD137, IL-2, TLR ligands, or STING), and/or a kinase inhibitor (*e.g.*, Braf inhibitor or MEK inhibitor)

Table 6 below provides exemplary genetic modifications for PD blockade, immune inhibitor, death receptor, immunostimulator, toll like receptor, kinase inhibition, master regulator, cytokine signaling, cell interaction reduction, and drug interaction related edits. The tables also provides target sequences for guide RNA using Cas9 in T cells as well as Genbank accession numbers for sequences that can be used for expression/overexpression. Genome editing using gRNAs is performed through transduction of lentivirus (lentiCRISPRv2) containing the desired gRNA and the *Streptococcus pyogenes* Cas9 nuclease. This can be perform as an alternative to delivery of TALEN RNA in the protocols. Sanjana, et al. *Nature Methods* (2014) 11(8):783-784.

Table 6. Exemplary Genes for Genetic Modification and Corresponding Cas9-Mediated Edits

PD blockade related edits	Genbank accession no.	Genomic sequences (gRNA) for Cas9 targeting
Programmed cell death protein 1 (PDCD1)	NP_005009.2	TGACGTTACCTCGTGCGGCC (SEQ ID NO: 146), CACGAAGCTCTCCGATGTGT (SEQ ID NO: 147), GCGTGACTTCCACATGAGCG (SEQ ID NO: 148), TTGGAAGTGGCCGGCTGGCC (SEQ ID NO: 149), GTGGCATACTCCGTCTGCTC (SEQ ID NO: 150),

		GATGAGGTGCCCCATTCCGCT (SEQ ID NO: 151),
Programmed cell death 1 ligand 1 (CD274)	NP_005009.2	TACCGCTGCATGATCAGCTA (SEQ ID NO: 152), AGCTACTATGCTGAACCTTC (SEQ ID NO: 153), GGATGACCAATTCAGCTGTA (SEQ ID NO: 154), ACCCCAAGGCCGAAGTCATC (SEQ ID NO: 155), TCTTTATATTCATGACCTAC (SEQ ID NO: 156), ACCGTTCAGCAAATGCCAGT (SEQ ID NO: 157)
Immune-inhibitor related edits	Genbank accession no.	Genomic sequences for Cas9 targeting
Cytotoxic T-lymphocyte protein 4 (CTLA4)	NP_005205.2	GTACCCACCGCCATACTACC (SEQ ID NO: 158), TTGCCTATGCCCAGGTAGTA (SEQ ID NO: 159), CCTTGTGCCGCTGAAATCCA (SEQ ID NO: 160), ACCCCGAACTAACTGCTGCA (SEQ ID NO: 161), ACATAGACCCCTGTTGTAAG (SEQ ID NO: 162), ATCCTTGCAGCAGTTAGTTC (SEQ ID NO: 163)
CTLA4-Ig (Orencia)	APZ76727.1	
Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform (PPP2CA)	NP_002706.1	ACATCGAACCTCTTGACGCT (SEQ ID NO: 164), TACAGCTCACCTTCTCGCAG (SEQ ID NO: 165), GGTATATCTCCTCGAGGAGC (SEQ ID NO: 166), TACACTGCTTGTAGCTCTTA (SEQ ID NO: 167), GAGCTCTAGACACCAACGTG (SEQ ID NO: 168), CAAGCAGCTGTCCGAGTCCC (SEQ ID NO: 169)
Serine/threonine-protein phosphatase 2A catalytic subunit beta isoform (PPP2CB)	CAA31183.1	AATGTGTAGCCAGCACCACG (SEQ ID NO: 170), GAACTTCCTGTAAACGATCC (SEQ ID NO: 171), TACATACCTCCATTACAAGC (SEQ ID NO: 172), CCATCTACTAAAGCTGTAAG

		(SEQ ID NO: 173), CTCAATATTGTAATGCGTTC (SEQ ID NO: 174), CTCTCCATCCATAGACACAC (SEQ ID NO: 175)
Protein tyrosine phosphatase, non-receptor type 6 (PTPN6)	AAA35963.1	TAAGACCTACATCGCCAGCC (SEQ ID NO: 176), GAAGAACTTGCACCAGCGTC (SEQ ID NO: 177), GTCAGCCGCATTCAACCCTCG (SEQ ID NO: 178), CTGCCAGAAGTCATTGACCG (SEQ ID NO: 179), CCCAGCCGTACTATGCCACG (SEQ ID NO: 180), GCCGCTGCCCTTCCAGACGC (SEQ ID NO: 181)
Tyrosine-protein phosphatase non-receptor type 22 (PTPN22)	AAD00904.1	GTAGCTGGAATCCTCATCAG (SEQ ID NO: 182), CAAAACCTATCCTACAACCTG (SEQ ID NO: 183), TTAGGGAGTTTATGGACCCA (SEQ ID NO: 184), CTCAGCCACAGTTGTAGGAT (SEQ ID NO: 185), TCACTGTACCTTAATGAAGT (SEQ ID NO: 186), TCCTTTATCTACAACCCTCC (SEQ ID NO: 187)
Lymphocyte activation gene 3 protein (LAG3)	CAA36243.3	TCCATAGGTGCCCAACGCTC (SEQ ID NO: 188), GTTCCGGAACCAATGCACAG (SEQ ID NO: 189), GCGAGAAGTCCCCGCGCTGC (SEQ ID NO: 190), TGACCCCTGCTCTTCGCAGA (SEQ ID NO: 191), CGCCGGCGAGTACCGCGCCG (SEQ ID NO: 192), TGGGCGGTCAGGGCGGCTGA (SEQ ID NO: 193)
Hepatitis A virus cellular receptor 2 (Tim3, HAVCR2)	AAM19100.1	CTAAATGGGGATTTCGCAA (SEQ ID NO: 194), ATCCCCATTTAGCCAGTATC (SEQ ID NO: 195), GTGAAGTCTCTCTGCCGAGT (SEQ ID NO: 196), AGGTCACCCCTGCACCGACT (SEQ ID NO: 197), CTTACTGTTAGATTTATATC

		(SEQ ID NO: 198), TATAGCAGAGACACAGACAC (SEQ ID NO: 199)
B- and T-lymphocyte attenuator (BTLA)	AAP44003.1	GTGACTTGGTGCAAGCTCAA (SEQ ID NO: 200), TCTGCTTGCCATTTTCGTCCT (SEQ ID NO: 201), CTGTTAGCACAGTATTTTAC (SEQ ID NO: 202), CCAAAGGAAGTAAACGATAC (SEQ ID NO: 203), ATGTTCCAGATGTCCAGATA (SEQ ID NO: 204), CTTCTTCTTAATCCCATATC (SEQ ID NO: 205)
CD160 antigen (CD160)	AAC72302.1	AGTTTAGTCGCGTTCCTTCC (SEQ ID NO: 206), CACTGTGCAACGGTGTGACT (SEQ ID NO: 207), GGATGTCCACAATTGCCAGC (SEQ ID NO: 208), AACTGAGAGTGCCTTCATTA (SEQ ID NO: 209), GACAGGGAACACACAGTGA (SEQ ID NO: 210), GACAGGGAACACACAGTGA (SEQ ID NO: 210), ATTGTGGACATCCAGTCTGG (SEQ ID NO: 212)
T-cell immunoreceptor with Ig and ITIM domains (TIGIT)	BAC04973.1	TCGCTGACCGTGAACGATAC (SEQ ID NO: 213), TGGGGCCACTCGATCCTTGA (SEQ ID NO: 214), GCAGATGACCACCAGCGTCG (SEQ ID NO: 215), TCAGGCCTTACCTGAGGCGA (SEQ ID NO: 216), CATCTGCACAGCAGTCATCG (SEQ ID NO: 217), ATTGAAGTAGTCATGCAGCT (SEQ ID NO: 218)
T-cell surface protein tactile (CD96)	AAA36662.1	AGGCACAGTAGAAGCCGTAT (SEQ ID NO: 219), GCTGTCTATCATCCCCAATA (SEQ ID NO: 220), ACTTACCACCGACCATGCAT (SEQ ID NO: 221)
Cytotoxic and regulatory T-cell molecule (CRTAM)	AAC80267.1	CACACTTTAGAGTGAGCGTC (SEQ ID NO: 222),

		CTCCAGTGGCTGACCCCCTC (SEQ ID NO: 223), CCACAGCAGCCCACCAGTAC (SEQ ID NO: 224)
Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1)	AF013249.1	TTATAATAGATGCAGCGATA (SEQ ID NO: 225), TCATTGTGACTGTTGTCCGA (SEQ ID NO: 226), GCCAGGCACCGTGATCCCCC (SEQ ID NO: 227)
Sialic acid-binding Ig-like lectin 7 (SIGLEC7)	AF170485.1	CATCCTTATCCCCGGTACCC (SEQ ID NO: 228), CAGAGAGTTCTGAGCTCGAC (SEQ ID NO: 229), AGTGTTGCTGGGGGCGGTCG (SEQ ID NO: 230)
Sialic acid-binding Ig-like lectin 9 (SIGLEC9)	AF135027.1	GACGATGCAGAGTTCCGTGA (SEQ ID NO: 231), ACTCACAGGACACGTTGAGA (SEQ ID NO: 232), TACCCTGGCCCAGTAGTTCA (SEQ ID NO: 233)
Natural killer cell receptor 2B4 (CD244)	AF105261.1	ACCTTCGTCTGTATGCTGTT (SEQ ID NO: 234), ACCAAACAGCATACAGACGA (SEQ ID NO: 235), CTACTCTATGATCCAGTCCC (SEQ ID NO: 236)
Death receptors and pathway edits		
Tumor necrosis factor ligand superfamily member 10 (TRAIL)	AAC50332.1	ACTCCGTCAGCTCGTTAGAA (SEQ ID NO: 237), GTTCACTCTCTTCGTCAT (SEQ ID NO: 238), AGAGTAGCAGCTCACATAAC (SEQ ID NO: 239)
Tumor necrosis factor receptor superfamily member 10B (TNFRSF10B)	AF018657.1	TTCCAGAGCTCACAACGACC (SEQ ID NO: 240), ATAGTCCTGTCCATATTTGC (SEQ ID NO: 241), AGATACTCACGATCTCATTG (SEQ ID NO: 242)
Tumor necrosis factor receptor superfamily member 10A (TNFRSF10A)	AAC51226.1	AGGTCAAGGATTGTACGCCC (SEQ ID NO: 243), GAAGTCCCTGCACCACGACC (SEQ ID NO: 244), TTTGGTTGTTCCGTTGCTGT

		(SEQ ID NO: 245)
Caspase-8 (CASP8)	CAA66853.1	TGATCGACCCTCCGCCAGAA (SEQ ID NO: 246), GGGTCGATCATCTATTAATA (SEQ ID NO: 247), TCCTTTGCGGAATGTAGTCC (SEQ ID NO: 248)
Caspase-10 (CASP10)	AAC50644.1	CTATGTATCCTTTCGGCATG (SEQ ID NO: 249), TCTTCTGCCGTATGATATAG (SEQ ID NO: 250), GTGAGACATGATCTCCCGAA (SEQ ID NO: 251)
Caspase-3 (CASP3)	AAA65015.1	ATGTCGATGCAGCAAACCTC (SEQ ID NO: 252), ATTATACATAAACCCATCTC (SEQ ID NO: 253), AATGGACTCTGGAATATCCC (SEQ ID NO: 254)
Caspase-6 (CASP6)	AAC50168.1	ATAGAGACAATCTTACCCGC (SEQ ID NO: 255), AAGATTGTCTCTATCTGCGC (SEQ ID NO: 256), AAATGTGATTGCCTTCGCCA (SEQ ID NO: 257)
Caspase-7 (CASP7)	AAC50303.1	CGTTTGTACCGTCCCTCTTC (SEQ ID NO: 258), TGCATCCATCAAGACCACC (SEQ ID NO: 259), TTGATATTTAGGCTTGCCGA (SEQ ID NO: 260)
FAS-associated death domain protein (FADD)	AAA86517.1	AGTCGTCGACGCGCCGCAGC (SEQ ID NO: 261), AGCGGCCCATCAGGACGCTT (SEQ ID NO: 262), GCGGCGCGTCGACGACTTCG (SEQ ID NO: 263)
Tumor necrosis factor receptor superfamily member 6 (FAS)	AAA63174.1	GTGTAACATACCTGGAGGAC (SEQ ID NO: 264), TACATCTGCACTTGGTATTC (SEQ ID NO: 265), CTAAAACTTACTTGGTGCAA (SEQ ID NO: 266)
IDO	AAA36081.1	TCTCAACTCTTTCTCGAAGC (SEQ ID NO: 267), CTGCCTGATCTCATAGAGTC

		(SEQ ID NO: 268), CAGATACTTACTCATAAGTC (SEQ ID NO: 269)
Arginase EIF2AK4	AAH09350.2	CGCTGAGAAATGACTGCACG (SEQ ID NO: 270), CATATACTTCTTCACCAGTT (SEQ ID NO: 271), ATGTACTCACACATCTGGAT (SEQ ID NO: 272)
Immunostimulator edits		
OX40 (TRAF2)	BAA14259.1	ACCGAATGTCCCGCGTGCAA (SEQ ID NO: 273), GCCTTTGCACGCGGGACATT (SEQ ID NO: 274), GGGGACCCTGAAAGAATACG (SEQ ID NO: 275)
CD137 (TNFRSF9)	TNFRSF9	CCTGCGCTGGAGAACTATT (SEQ ID NO: 276), CCTTGTAGTAACTGCCCAGC (SEQ ID NO: 277), CATAGTAGCCACTCTGTTGC (SEQ ID NO: 278)
IL2	CAA25292.1	CAATATCAACGTAATAGTTC (SEQ ID NO: 279), GACTTAGTGCAATGCAAGAC (SEQ ID NO: 280), GATATTGCTGATTAAGTCCC (SEQ ID NO: 281)
Stimulator of interferon genes protein (STING or TMEM173)	ACI46648.1	GCGGGCCGACCGCATTTGGG (SEQ ID NO: 282), CATATTACATCGGATATCTG (SEQ ID NO: 283), ACTCTTCTGCCGGACACTTG (SEQ ID NO: 284)
Toll like receptor edits		
TLR1	AAC34137.1	TTATAGAGGAACCCTACTAA (SEQ ID NO: 285), TTGTGGGCACCTTACTGAGT (SEQ ID NO: 286), CGAACACATCGCTGACAACT (SEQ ID NO: 287)
TLR2	AAC34377.1	GTTAACGTTTCCACTTTACC (SEQ ID NO: 288), TTCCCGCTGAGCCTCGTCCA (SEQ ID NO: 289),

		TATCTAATTTATCGTCTTCC (SEQ ID NO: 290)
TLR3	AAC34134.1	TTCGGAGCATCAGTCGTTGA (SEQ ID NO: 291), TTCAACGACTGATGCTCCGA (SEQ ID NO: 292), CATGCACTCTGTTTGCGAAG (SEQ ID NO: 293)
TLR4	AAC80227.1	TTCTCCCAGAACCAAACGA (SEQ ID NO: 294), GATGATGTCTGCCTCGCGCC (SEQ ID NO: 295), ATGCCCCATCTTCAATTGTC (SEQ ID NO: 296)
TLR5	AAC34376.1	TATTCGGCCATCAAAGGAGC (SEQ ID NO: 297), GACTAAGCCTCAACTCCAAC (SEQ ID NO: 298), TATACAAGCTATTAGCTGCG (SEQ ID NO: 299)
TLR6	BAA78631.1	GAACTACATCGCTGAGCTTC (SEQ ID NO: 300), GCCATCCTATTGTGAGTTTC (SEQ ID NO: 301), TGTCTCCAATTAACTAACG (SEQ ID NO: 302)
TLR7	AAF60188.1	AAGGAATAGTCACCTCCGTA (SEQ ID NO: 303), AATGGGGCATTATAACAACG (SEQ ID NO: 304), GGTGAGGTTCGTGGTGTTCG (SEQ ID NO: 305)
TLR8	AAF64061.1	GTGCAGCAATCGTCGACTAC (SEQ ID NO: 306), AATCCCGGTATACAATCAAA (SEQ ID NO: 307), CTCGAGTTGCTTGACTTACG (SEQ ID NO: 308)
TLR9	AAF72189.1	GGCTCACGGCTATTCGGCCG (SEQ ID NO: 309), GCGTCTCCGTGACAATTACC (SEQ ID NO: 310), CCGACAGGTCCACGTAGCGC (SEQ ID NO: 311)
TLR10	AAK26744.1	CCCACATTTACGCCTATCCT (SEQ ID NO: 312),

		TAACATTAATAGCAGCTCGA (SEQ ID NO: 313), GACCCCAGCCACAACGACAC (SEQ ID NO: 314)
Kinase inhibition edits		
Serine/threonine-protein kinase B-raf (BRAF)	AAA35609.2	CCCCACCAAATTTGTCCAAT (SEQ ID NO: 315), GAGGCCCTATTGGACAAATT (SEQ ID NO: 316), GTTGCTCCGTGCCACATCTG (SEQ ID NO: 317)
Dual specificity mitogen-activated protein kinase kinase 1 (MAP2K or MEK)	AAA36318.1	CCATACTTACTCCGCAGAGC (SEQ ID NO: 318), TATGGTGCGTTCTACAGCGA (SEQ ID NO: 319), CCCGACGGCTCTGCAGTTAA (SEQ ID NO: 320)
Master regulator edits		
FoxP3	AAG53607.1	
Cytokine signaling edits (The goal of one or both of these edits is to prevent or minimize conversion of therapeutic Tregs into Th17 cells due to endogenous IL-6. Gagliani et al. <i>Nature</i> , 523(7559):221- 225 2015. Korn et al. <i>Proceedings of the National Academy of Sciences</i> , 105(47):18460- 18465, 2008.		
Interleukin-6 receptor subunit alpha (IL6R)	CAA31312.1	TCGGTGCAGCTCCACGACTC (SEQ ID NO: 321), AACTATTCATGCTACCGGGC (SEQ ID NO: 322), CGTGGTGCAGCTTCGTGCCC (SEQ ID NO: 323)
Interleukin-6 receptor subunit beta (IL6ST or GP130)	AAA59155.1	AGATGCCTCAACTTGGAGCC (SEQ ID NO: 324), TTTGAGTTGCATTGTGAACG (SEQ ID NO: 325), ATTCGCTGTATGAAGGAAGA (SEQ ID NO: 326)
Cell interaction reducing edits		
TCR alpha (see preferred talen edit)		

TCR beta (see preferred talen edit)		
CIITA	CAA52354.1	TTCCTACACAATGCGTTGCC (SEQ ID NO: 327), GATATTGGCATAAGCCTCCC (SEQ ID NO: 328), TCAACTGCGACCAGTTCAGC (SEQ ID NO: 329)
B7-1(CD80)	AAA36045.1	TCGTATGTGCCCTCGTCAGA (SEQ ID NO: 330), GAGTGAATCAGACCTTCAAC (SEQ ID NO: 331), TATGGCCCGAGTACAAGAAC (SEQ ID NO: 332)
B7-2(CD86)	AAB03814.1	GTAACCGTGTATAGATGAGC (SEQ ID NO: 333), ATACTCGATAGTTGAATTCT (SEQ ID NO: 334), CATCAGATCTTTCAGGTATA (SEQ ID NO: 335)
b2m	AAA51811.1	ACTCACGCTGGATAGCCTCC (SEQ ID NO: 336), GAGTAGCGCGAGCACAGCTA (SEQ ID NO: 337), CAGTAAGTCAACTTCAATGT (SEQ ID NO: 338)
UL18	CAA68399.1	
PDL2	AAK31105.1	
FasL	AAC50071.1	GGTTGTTGCAAGATTGACCC (SEQ ID NO: 339), GAGGAACTCTAAGTATCCCC (SEQ ID NO: 340), TCTGGTTGCCTTGGTAGGAT (SEQ ID NO: 341)
Perforin (PRF1)	CAA31612.1	CGCAGCCACAAGTTCGTGCC (SEQ ID NO: 342), GGAGCTGGGTGGCCGCATAT (SEQ ID NO: 343), CCCGAACAGCAGGTCGTAA (SEQ ID NO: 344)
Galectin 9 (LGALS9)	AA88922.1	
PVT/CD155	AAA36461.1	

Drug interaction related edits		
CD52	CAA44323.1	CTCTTACCTGTACCATAACC (SEQ ID NO: 345), AATGCCTCCGCTTATGTTGC (SEQ ID NO: 346), TGGCATTGGCCACGAAGAAA (SEQ ID NO: 347)
tocilizumab-like heavy chain (for scfv)	BAJ21229.1	
tocilizumab-like light chain (for scfv)	BAJ21230.1	
Integrin alpha-4 (ITGA4)	CAA34852.1	CGACTACTTCGGTAGTATGC (SEQ ID NO: 348), CAGCATACTACCGAAGTAGT (SEQ ID NO: 349), GTGTTTGTGTACATCAACTC (SEQ ID NO: 350)

The table below provides target sequences for TALEN edits as well as protein sequences.

Table 7. Primarily TALEN mediated edits

CS1 edit related	Sequence or Genbank Accession no.
CS1	NP_067004.3
CS1v1TALEN target	tgacttccagagag caatatgget ggttccceaa catgcctca (SEQ ID NO: 351)
CS1v1 left TALEN target	tgacttccagagagcaa (SEQ ID NO: 352)
CS1v1 left TALEN protein	MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKKVGIHGVPAAVDLRTLGYSSQQQEKIKPK VRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQ WSGARALEALLTVAGELRGPPLQLDTGQLLKIARGGVTAVEAVHAWRNALTGAPLNLT EQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLC QAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQ RLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGGK QALETVQRLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAI ASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLT PEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLC QAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQ RLLPVLCQAHGLTPEQVVAIASHDGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQ ALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAI CLGCRPALDAVKKGLPHAPALIKRTNRRIPERTSHRVAGSQLVKSELEKKSELRHKLKYVP HEYIELJEIARNSTQDRILEMKVMEFFMKVYGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDT KAYSGGYNLPQGADEMORYVEENQTRNKHINPNEWKVPSSVTEFKFLFVSGHFKGNV KAQLTRLNHNITNCNCAVLSVEELLIGGEMIKAGTLTLEEVRKFNNGEINFERS (SEQ ID NO: 353)
CS1v1 right TALEN target	aacatgcctc accctca (SEQ ID NO: 354)
CS1v1 right TALEN protein	MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKKVGIHGVPAAVDLRTLGYSSQQQEKIKPK VRSTVAQHHEALVGHGFTHAHIVALSQHPAALGTAVKYQDMIAALPEATHEAIVGVGKQ WSGARALEALLTVAGELRGPPLQLDTGQLLKIARGGVTAVEAVHAWRNALTGAPLNLT EQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLC QAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQ

[illegible]

[illegible]

[illegible]

[illegible]

	LCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNIGGKQALET VQALLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDG GKQALETVQRLLPVLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPQQV VAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASNNGGKQALETVQRLLPVLCQAHG LTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGKQALETVQRLLP VLCQAHGLTPQQVVAIASNNGGKQALETVQRLLPVLCQAHGLTPEQVVAIASHDGKQALETVQRLLP AVKKGLGDPISRSQVVKSELEKKSELRHKLKYVPHEYIELIEIARNSTQDRILEMKVMEFFM KVGGRGKHLGGRKPDGAIYTVGSPIDYGVIVDTKAYSGGYNLPQGADEMQRVVEENQT RNKHINPNEWVKVYPSSVTEFKFLFVSGHFKGNYKAQLTRLNHTNCGAVLSVEELLIGGE MIKAGTLTLEEVRKFNNGEINFAAD (SEQ ID NO: 383)
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(iii) Exemplary Genetic Modification Approaches

Any conventional genetic modification approaches can be used to genetically modify the immune cells in a manner as described herein. In some embodiments, the genetic modification is performed using genome editing. “Genome editing” refers to a method of modifying the genome, including any protein-coding or non-coding nucleotide sequence, of an organism to knock out the expression of a target gene. In general, genome editing methods involve use of an endonuclease that is capable of cleaving the nucleic acid of the genome, for example at a targeted nucleotide sequence. Repair of the double-stranded breaks in the genome may be repaired introducing mutations and/or exogenous nucleic acid may be inserted into the targeted site.

Genome editing methods are generally classified based on the type of endonuclease that is involved in generating double stranded breaks in the target nucleic acid. These methods include use of zinc finger nucleases (ZFN), transcription activator-like effector-based nuclease (TALEN), meganucleases, and CRISPR/Cas systems.

In some instances, genetic modification of the immune cells as described herein is performed using the TALEN technology known in the art. TALENs are engineered restriction enzymes that can specifically bind and cleave a desired target DNA molecule. A TALEN typically contains a Transcriptional Activator-Like Effector (TALE) DNA-binding domain fused to a DNA cleavage domain. The DNA binding domain may contain a highly conserved 33-34 amino acid sequence with a divergent 2 amino acid RVD (repeat variable dipeptide motif) at positions 12 and 13. The RVD motif determines binding specificity to a nucleic acid sequence and can be engineered according to methods known to those of skill in the art to specifically bind a desired DNA sequence (see, *e.g.*, Juillerat, et al. (January 2015). Scientific reports, 5; Miller et al. (February 2011). Nature Biotechnology 29 (2): 143–8; Zhang et al. (February 2011). Nature Biotechnology 29 (2): 149–53; Geißler, et al., Boch, (2011), PLoS ONE 6 (5): e19509; Boch (February 2011). Nature Biotechnology 29 (2): 135–6; Boch, et al. (December 2009). Science 326 (5959): 1509–12; and Moscou et al,

(December 2009). *Science* 326 (5959): 1501. The DNA cleavage domain may be derived from the FokI endonuclease, which is active in many different cell types. The FokI domain functions as a dimer, requiring two constructs with unique DNA binding domains for sites in the target genome with proper orientation and spacing. Both the number of amino acid residues between the TALE DNA binding domain and the FokI cleavage domain and the number of bases between the two individual TALEN binding sites appear to be important parameters for achieving high levels of activity. Miller et al. (2011) *Nature Biotech.* 29: 143-8.

TALENs specific to sequences in a target gene of interest (*e.g.*, TCR, CD52, MHC, and others described herein) can be constructed using any method known in the art, including various schemes using modular components. Zhang et al. (2011) *Nature Biotech.* 29: 149-53; Geibler et al. (2011) *PLoS ONE* 6: e19509.

A TALEN specific to a target gene of interest can be used inside a cell to produce a double-stranded break (DSB). A mutation can be introduced at the break site if the repair mechanisms improperly repair the break *via* non-homologous end joining. For example, improper repair may introduce a frame shift mutation. Alternatively, a foreign DNA molecule having a desired sequence can be introduced into the cell along with the TALEN. Depending on the sequence of the foreign DNA and chromosomal sequence, this process can be used to correct a defect or introduce a DNA fragment into a target gene of interest, or introduce such a defect into the endogenous gene, thus decreasing expression of the target gene.

In some instances, genetic modification of the immune cells as described herein is performed using CRISPR technology as known in the art (CRISPR/Cas systems). Such modification may include the deletion or mutation of a sequence in a target gene of interest can be constructed using a CRISPR-Cas system, where the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas system is an engineered, non-naturally occurring CRISPR-Cas system. The present disclosure utilizes the CRISPR/Cas system that hybridizes with a target sequence in a target gene of interest, where the CRISPR/Cas system comprises a Cas endonuclease and an engineered crRNA/tracrRNA (or single guide RNA ("sgRNA")). In some embodiments, the CRISPR/Cas system includes a crRNA and does not include a tracrRNA sequence. CRISPR/Cas complex can bind to the lineage specific protein polynucleotide and allow the cleavage of the protein polynucleotide, thereby modifying the polynucleotide.

The CRISPR/Cas system of the present disclosure may bind to and/or cleave the region of interest within a target gene of interest, within or adjacent to the gene, such as, for example, a leader sequence, trailer sequence or intron, or within a non-transcribed region, either upstream or downstream of the coding region. The guide RNAs (gRNAs) used in the present disclosure may be designed such that the gRNA directs binding of the Cas enzyme-gRNA complexes to a pre-determined cleavage sites (target site) in a genome. The cleavage sites may be chosen so as to release a fragment that contains a region of unknown sequence, or a region containing a SNP, nucleotide insertion, nucleotide deletion, rearrangement, etc. Cleavage of a gene region may comprise cleaving one or two strands at the location of the target sequence by the Cas enzyme. In one embodiment, such, cleavage can result in decreased transcription of a target gene. In another embodiment, the cleavage can further comprise repairing the cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide, wherein the repair results in an insertion, deletion, or substitution of one or more nucleotides of the target polynucleotide.

The terms “gRNA,” “guide RNA” and “CRISPR guide sequence” may be used interchangeably throughout and refer to a nucleic acid comprising a sequence that determines the specificity of a Cas DNA binding protein of a CRISPR/Cas system. A gRNA hybridizes to (complementary to, partially or completely) a target nucleic acid sequence in the genome of a host cell. The gRNA or portion thereof that hybridizes to the target nucleic acid may be between 15-25 nucleotides, 18-22 nucleotides, or 19-21 nucleotides in length. In some embodiments, the gRNA sequence that hybridizes to the target nucleic acid is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In some embodiments, the gRNA sequence that hybridizes to the target nucleic acid is between 10-30, or between 15-25, nucleotides in length.

In addition to a sequence that binds to a target nucleic acid, in some embodiments, the gRNA also comprises a scaffold sequence. Expression of a gRNA encoding both a sequence complementary to a target nucleic acid and scaffold sequence has the dual function of both binding (hybridizing) to the target nucleic acid and recruiting the endonuclease to the target nucleic acid, which may result in site-specific CRISPR activity. In some embodiments, such a chimeric gRNA may be referred to as a single guide RNA (sgRNA).

As used herein, a “scaffold sequence,” also referred to as a tracrRNA, refers to a nucleic acid sequence that recruits a Cas endonuclease to a target nucleic acid bound (hybridized) to a complementary gRNA sequence. Any scaffold sequence that comprises at

least one stem loop structure and recruits an endonuclease may be used in the genetic elements and vectors described herein. Exemplary scaffold sequences will be evident to one of skill in the art and can be found, for example, in Jinek, et al. *Science* (2012) 337(6096):816-821, Ran, et al. *Nature Protocols* (2013) 8:2281-2308, PCT Application No. WO2014/093694, and PCT Application No. WO2013/176772. In some embodiments, the CRISPR-Cas system does not include a tracrRNA sequence.

In some embodiments, the gRNA sequence does not comprise a scaffold sequence and a scaffold sequence is expressed as a separate transcript. In such embodiments, the gRNA sequence further comprises an additional sequence that is complementary to a portion of the scaffold sequence and functions to bind (hybridize) the scaffold sequence and recruit the endonuclease to the target nucleic acid.

In some embodiments, the gRNA sequence is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or at least 100% complementary to a target nucleic acid (see also U.S. Patent 8,697,359, which is incorporated by reference for its teaching of complementarity of a gRNA sequence with a target polynucleotide sequence). It has been demonstrated that mismatches between a CRISPR guide sequence and the target nucleic acid near the 3' end of the target nucleic acid may abolish nuclease cleavage activity (Upadhyay, et al. *Genes Genome Genetics* (2013) 3(12):2233-2238). In some embodiments, the gRNA sequence is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or at least 100% complementary to the 3' end of the target nucleic acid (e.g., the last 5, 6, 7, 8, 9, or 10 nucleotides of the 3' end of the target nucleic acid).

Example sgRNA sequences, including both modified and unmodified sgRNAs, targeting the T cell receptor alpha constant (TRAC) gene are provided herein. As will be evident to one of ordinary skill in the art, selection of sgRNA sequences may depend on factors such as the number of predicted on-target and/or off-target binding sites. In some embodiments, the sgRNA sequence is selected to maximize potential on-target and minimize potential off-target sites.

In some embodiments, the Cas endonuclease is a Cas9 nuclease (or variant thereof) or a Cpf1 nuclease (or variant thereof). Cas9 endonucleases cleave double stranded DNA of a target nucleic acid resulting in blunt ends, whereas cleavage with Cpf1 nucleases results in staggered ends of the nucleic acid.

In general, the target nucleic acid is flanked on the 3' side or 5' side by a protospacer adjacent motif (PAM) that may interact with the endonuclease and be further

involved in targeting the endonuclease activity to the target nucleic acid. It is generally thought that the PAM sequence flanking the target nucleic acid depends on the endonuclease and the source from which the endonuclease is derived. For example, for Cas9 endonucleases that are derived from *Streptococcus pyogenes*, the PAM sequence is NGG, although the PAM sequences NAG and NGA may be recognized with lower efficiency. For Cas9 endonucleases derived from *Staphylococcus aureus*, the PAM sequence is NNGRRT. For Cas9 endonucleases that are derived from *Neisseria meningitidis*, the PAM sequence is NNNNGATT. Cas9 endonucleases derived from *Streptococcus thermophilus*, St1Cas9 and dSt3Cas9, the PAM sequences are NNAGAAW and NGGNG, respectively. For Cas9 endonuclease derived from *Treponema denticola*, the PAM sequence is NAAAAC. In some embodiments, the Cas endonuclease is a Cpf1 nuclease. In contrast to Cas9 endonucleases, Cpf1 endonuclease generally do not require a tracrRNA sequence and recognize a PAM sequence located at the 5' end of the target nucleic acid. For a Cpf1 nuclease, the PAM sequence is TTTN. In some embodiments, the Cas endonuclease is MAD7 (also referred to as Cpf1 nuclease from *Eubacterium rectale*) and the PAM sequence is YTTTN.

In some embodiments, genetically engineering a cell also comprises introducing a Cas endonuclease, or nucleic acid sequence encoding such (*e.g.*, mRNA encoding a Cas endonuclease), into the cell. In some embodiments, the Cas endonuclease and the nucleic acid encoding the gRNA are provided on the same nucleic acid (*e.g.*, a vector). In some embodiments, the Cas endonuclease and the nucleic acid encoding the gRNA are provided on different nucleic acids (*e.g.*, different vectors). In some embodiments, the Cas endonuclease is provided as an mRNA encoding the Cas endonuclease and the gRNA is provided as a modified gRNA molecule. Alternatively or in addition, the Cas endonuclease may be provided or introduced into the cell in protein form.

In some embodiments, the Cas endonuclease is a Cas9 enzyme or variant thereof. In some embodiments, the Cas9 endonuclease is derived from *Streptococcus pyogenes*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Streptococcus thermophilus*, or *Treponema denticola*. In some embodiments, the nucleotide sequence encoding the Cas endonuclease may be codon optimized for expression in a host cell. In some embodiments, the endonuclease is a Cas9 homolog or ortholog.

In some embodiments, the nucleotide sequence encoding the Cas9 endonuclease is further modified to alter the activity of the protein. In some embodiments, the Cas9

endonuclease has been modified to inactivate one or more catalytic residues of the endonuclease. In some embodiments, the Cas9 endonuclease has been modified to inactivate one of the catalytic residues of the endonuclease, referred to as a “nickase” or “Cas9n”. Cas9 nickase endonucleases cleave one DNA strand of the target nucleic acid. In some embodiments, the methods described herein involve two distinct cleavage reactions, in which one Cas9 nickase is directed to cleave one DNA strand of the target nucleic acid and a Cas9 nickase is directed to cleave the second DNA strand of the target nucleic acid.

(iv) *MHC-CAR Regulatory T cells (Treg)*

Any of the MHC-CAR-expressing T cells disclosed herein can be regulatory T cells (Treg), which may mimic the immune modulation activity of follicular regulatory cells. As used herein, regulatory T cells or Treg cells, which are also known as suppressor T cells, refer to a subpopulation of T cells that modulate the immune system, maintain tolerance to self-antigens, and/or prevent autoimmune disease. Treg cells function as immunesuppressors to suppress or downregulate induction and/or proliferation of effector T cells, such as pathologic CD4⁺ and/or CD8⁺ cells involved in autoimmune diseases.

The genetically modified Treg cells disclosed herein express one or more of the biomarkers associated with Treg cells in nature, for example, CD4, FOXP3, CD25, CD45R (*e.g.*, CD45RA or CD45RO), or a combination thereof. The Treg cells may be prepared using (derived from) peripheral blood mononuclear cells (PBMCs) isolated from a suitable donor (*e.g.*, the human patient subject to the treatment). Methods for isolating the subpopulation of Treg cells from PBMCs are well known in the art, for example, cell sorting. Expression vectors for a suitable MHC-CAR construct, as well as other genetic modification (*e.g.*, those described herein) can be introduced into the Treg subpopulation via methods as described herein, or other methods known in the art. Alternatively, the genetically modified Treg cells may be prepared by introducing a transgene coding for CD25 and/or other Treg cell markers into suitable T cells, which can be further modified to introduce the expression cassette for the MHC-CAR and optionally other genetic modification as described herein.

In some embodiments, the genetically modified Treg cells may be further modified to display (*e.g.*, surface express or surface attach) molecules targeting a specific type of pathologic cells (*e.g.*, CD4⁺ cells or CD8⁺ cells) and/or display molecules targeting a specific tissue site (*e.g.*, lymph node or an inflammation site).

In some examples, the genetically modified Treg cells further express a chimeric

receptor (CAR) comprising an extracellular domain such as a single-chain antibody (scFv) specific to a B cell surface marker, for example, CD19. Alternatively or in addition, the Treg cells may further express a chimeric receptor comprising an extracellular domain (*e.g.*, scFv) specific to a T cell surface marker, for example, CS-1. Such a chimeric receptor can be a cell-surface receptor comprising an extracellular domain, a transmembrane domain, and a cytoplasmic domain (*e.g.*, comprising a co-stimulatory domain, a cytoplasmic signaling domain such as CD3 ζ , or a combination thereof) in a combination that is not naturally found together on a single protein.

The Treg cell may further display a molecule targeting lymph nodes and/or germinal center, for example, CXCR5, and/or display a molecule targeting an inflammation site, for example, CCR6. Targeting germinal center B cells (GC B cells) may be mediated, at least in part, by a specialized helper T cell subset, the CXCR5^{high}PD-1^{high} T follicular helper (TFH) cells. Foxp3⁺ Treg can be diverted to become TFH repressors *via* expression of Bcl6 and SAP-mediated interaction with B cells. The resulting follicular regulatory T cells (TFR) are expected to share features of both TFH and Treg cells, localize to germinal centers, and regulate the size of the TFH cell population and germinal centers *in vivo*.

Further, the Treg cells disclosed herein may include one or more of the additional genetic modification as described herein, for example, checkpoint molecule knock out.

The Treg cells expressing B-cell or T-cell specific CAR may target pathologic B cells and/or T cells involved in an autoimmune disease. For example, the genetically modified Treg cells as described herein would be expected to exhibit functions similar to follicular regulatory cells, *e.g.*, targeting B cells, T cells, and/or dendritic cells, thereby, *e.g.*, downregulating B cell stimulation, secreting suppressive cytokines that can inhibit activation of germinal center (GCB cells (such as IL-10 and TGF- β), inducing cytotoxicity of Tfh (through MHC CAR) and GC) B (*e.g.*, through the CD19 CAR), and/or mechanical disrupting signaling transduction to GC B cells or to T follicular helper (Tfh) cells (*e.g.*, through binding to GC B and MHC-peptide Tfh). Alternatively or in addition, the Treg cells may potentially engage both helper T cells, B cells, and/or antigen presenting cells, or in some instances, physically blocking the engagement.

III. Application of Immune Cells Expressing MHC-CAR in Immunotherapy

Host immune cells expressing MHC-CAR (the encoding nucleic acids or vectors comprising such) described herein are useful for targeting and eliminating pathogenic cells

involved in autoimmune diseases, such as MS, type I diabetes, lupus, rheumatoid arthritis, *etc.* In some embodiments, the subject is a mammal, such as a human, monkey, mouse, rabbit, or domestic mammal. In some embodiments, the subject is a human, for example, a human patient having, suspected of having, or at risk for an autoimmune disease (*e.g.*, MS).

The MHC-CAR-expressing immune cells can be mixed with a pharmaceutically acceptable carrier to form a pharmaceutical composition, which is also within the scope of the present disclosure. To perform the methods described herein, an effective amount of the immune cells expressing any of the MHC-CAR constructs described herein can be administered into a subject in need of the treatment. The immune cells may be autologous to the subject, *i.e.*, the immune cells are obtained from the subject in need of the treatment, genetically engineered for expression of the MHC-CAR constructs and optionally contains one or more of the additional genetic modifications as described herein, and then administered to the same subject. Administration of autologous cells to a subject may result in reduced rejection of the immune cells as compared to administration of non-autologous cells. Alternatively, the immune cells are allogeneic cells, *i.e.*, the cells are obtained from a first subject, genetically engineered for expression of the MHC-CAR construct, and administered to a second subject that is different from the first subject but of the same species. For example, allogeneic immune cells may be derived from a human donor and administered to a human recipient who is different from the donor.

In some embodiments, the immune cells are co-used with a therapeutic agent for the target immune disease, for example, Alemtuzumab for treating MS. Such immunotherapy is used to treat, alleviate, or reduce the symptoms of the target immune disease for which the immunotherapy is considered useful in a subject.

The efficacy of the MHC-CAR immunotherapy may be assessed by any method known in the art and would be evident to a skilled medical professional. For example, the efficacy of the immunotherapy may be assessed by survival of the subject and/or reduction of disease symptoms in the subject.

In some embodiments, the immune cells expressing any of the MHC-CAR disclosed herein are administered to a subject who has been treated or is being treated with a therapeutic agent for an autoimmune disease. The immune cells expressing any one of the MHC-CAR disclosed herein may be co-administered with the therapeutic agent. For example, the immune cells may be administered to a human subject simultaneously with the therapeutic agent. Alternatively, the immune cells may be administered to a human subject

during the course of a treatment involving the therapeutic agent. In some examples, the immune cells and the therapeutic agent can be administered to a human subject at least 4 hours apart, *e.g.*, at least 12 hours apart, at least 1 day apart, at least 3 days apart, at least one week apart, at least two weeks apart, or at least one month apart.

To practice the method disclosed herein, an effective amount of the immune cells expressing MHC-CAR or compositions thereof can be administered to a subject (*e.g.*, a human MS patient) in need of the treatment via a suitable route, such as intravenous administration. Any of the immune cells expressing MHC-CAR or compositions thereof may be administered to a subject in an effective amount. As used herein, an effective amount refers to the amount of the respective agent (*e.g.*, the immune cells expressing MHC-CAR or compositions thereof) that upon administration confers a therapeutic effect on the subject. Determination of whether an amount of the cells or compositions described herein achieved the therapeutic effect would be evident to one of skill in the art. Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. In some embodiments, the effective amount alleviates, relieves, ameliorates, improves, reduces the symptoms, or delays the progression of any disease or disorder in the subject. In some embodiments, the subject is a human. In some embodiments, the subject is a human cancer patient.

In some embodiments, the subject is a human patient suffering from an autoimmune disease, which is characterized by abnormal immune responses attacking a normal body part. Examples of autoimmune diseases include multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis (also known as juvenile idiopathic arthritis), Sjögren's syndrome, systemic sclerosis, ankylosing spondylitis, Type 1 diabetes, autoimmune thyroid diseases (Grave's and Hashimoto's), multiple sclerosis myasthenia gravis, inflammatory bowel disease (Crohn's or ulcerative colitis), Psoriasis, or a diseases mentioned in Table 1.

There are numerous stages in the immune cascade where, in general, an autoimmune disease can be impacted. There is a continuum of interventions made possible by the combination of edits that the methods disclosed herein comprises. For example, Treg cells displaying a distinct set of surface molecules, in addition to the MHC-CAR, can be used for

treating the autoimmune disease at different stages.

In the early stage of many immune disorders, including MS, there exist unexplained deficits in regulatory mechanisms and/or tolerance induction exists in MS and there begin repeated attacks on the nervous system by T cells. Treg cells expressing a suitable MHC-CAR and anti-CD19 CAR (optionally with other genetic modifications as described herein) may be used for intervention.

An advantage of autoreactive Treg cells is their ability to act as “bystander” suppressors, to dampen inflammation at a site-specific manner in response to cognate antigen expressed locally by affected tissues. The induction of regulatory T cells (by autoantigens) can suppress disease progression even when there are a variety of autoantigens (or when the initiating/primary) autoantigen is unknown. Tregs can travel relatively freely, and inhibit T cells and B cells and prevent return to an inflammatory environment. These autoreactive Tregs are advantageous in their ability to act as bystander suppressors and dampen inflammation in a site-specific manner in response to cognate antigen expressed locally by affected tissues.

Thus, the genetically modified Treg cells may be designed to mimic the suppressor function of the autoreactive Treg cells. Such Treg cells may be modified, for example, to have PD-L1/PD-1 knocked out, to display CCR6 and/or scFv targeting MOG to route to the site of inflammation, to express a suitable MHC-CAR and/or anti-CD19-CAR. Alternatively, the Treg cells may be modified, for example, to have PD-L1/PD-1 knocked out, to display CXCR5 to route to germinal centers and/or ectopic lymph nodes, to express a suitable MHC-CAR and/or anti-CD19-CAR. These types of Treg cells may interact with pathogenic cells at the site of inflammation, block pathogenic interactions, and/or calm inflammatory environment. They can be used at an early disease stage (to inhibit pathogenicity) or after cytotoxic therapy (to prevent return to an inflammatory environment).

Relapsing-remitting MS (mid-stage) naturally regulates itself, and treatments which augment these natural regulatory mechanisms will help control the disease process. In successful disease treatment, there is a shift from Th1 cells to Th2 and Th3 cells, and the appearance of other regulatory cells. At this stage, therapeutic targets will include both pathogenic B and pathogenic T cells. Treg cells for treating such mid-stage disease may express a suitable MHC-CAR as described herein, and an additional CAR targeting B cells (*e.g.*, an anti-CD19 CAR) or targeting T cells such as CD8+ cells. The Treg cells may further display CXCR5 or free of CXCR5 targeting. Treg cells expressing anti-CD19 CAR may be

used to eliminate B cells in the germinal center.

When MS changes from relapsing remitting to the chronic progressive form (late-stage), T cells enter a state of chronic activation and degenerative processes occur. Aggressive treatment against cytotoxic CD8⁺ T cells requires a CAR augmentation that is sufficiently cytotoxic. At this point, a treatment may shift from one primarily driven by Treg cells to one driven by MHC-CAR CD8⁺ T cells and even MHC-CAR CS-1 cells. The ultimate goal remains the same: to suppress pathology through cytotoxicity enhanced by bystander effect. Genetically engineered T cells for use at this disease stage may express a suitable MHC-CAR, and an additional CAR targeting pathologic T cells involved in the late stage of the disease, for example, CD8⁺ T cells. In some examples, the additional CAR may target CS-1 (also known as SLAMF7), which is a glycoprotein expressed on CD8⁺ T cells. CS1 is a promising antigen that can be used to target and kill CD8⁺ T cells and plasma cells. CS1-CAR T cells secrete more IFN-gamma as well as IL-2, expressing higher levels of activation marker CD69, higher capacity for degranulation, and display enhanced cytotoxicity. Anti-CS1 CAR will target CD8⁺ T-cells. The genetically modified T cells may further display a molecule for bone-marrow targeting of plasma cells, such as CXCR4, and their targeting to inflamed tissues, with CXCR3.

Hiepe et al., Nature Reviews Rheumatology, 7(3):170-178, 2011. Examples include targeting of plasma cells in lupus.

IV. Kits for Therapeutic Uses

The present disclosure also provides kits for use of the MHC-CAR-expressing immune cells for use in suppressing pathogenic immune cells such as autoreactive T cells in autoimmunity. Such kits may include one or more containers comprising compositions comprising immune cells expressing MAR-CAR such as those described herein), and a pharmaceutically acceptable carrier.

In some embodiments, the kit can comprise instructions for use in any of the methods described herein. The included instructions can comprise a description of administration of the MHC-CAR-expressing immune cells to a subject who needs the treatment, *e.g.*, an MS patient. The kit may further comprise a description of selecting a subject suitable for treatment based on identifying whether the subject is in need of the treatment. In some embodiments, the instructions comprise a description of administering the immune cells to a subject who is in need of the treatment.

The instructions relating to the use of the immune cells expressing the MHC-CAR described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the disclosure are typically written instructions on a label or package insert. The label or package insert indicates that the pharmaceutical compositions are used for treating, delaying the onset, and/or alleviating a disease or disorder in a subject.

The kits provided herein are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging, and the like. Also contemplated are packages for use in combination with a specific device. A kit may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port. At least one active agent in the pharmaceutical composition is immune cells expressing MHC-CAR as described herein.

Kits optionally may provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiment, the disclosure provides articles of manufacture comprising contents of the kits described above.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

The instant examples focuses on the development of a cellular immunotherapy utilizing chimeric receptors to selectively redirect therapeutic T cells against myelin basic protein (MBP)-specific T lymphocytes implicated in MS [16]. The results of this program can support the further development of redirected therapeutic T cells able to counteract pathologic, self-specific T lymphocytes, and specifically validate humanized MBP-DR2-chimeric receptors as a therapeutic target in MS [29].

Example 1: Construction of Modified T-cells Specific to Myelin Basic Protein

A construct for an antigen-specific T-cell receptor (TCR) that targets a MBP-loaded major histocompatibility complex-chimeric antigen receptor (MHC-CAR) is designed for reintroduction into cells with TCR knockouts for assays. Design of the TCR constructs is based on an antigen-specific TCR with a published structure and TCR expression constructs validated in human cell lines [17, 46, 51, 52]. Green fluorescent protein (eGFP) or luciferase is genetically encoded for labeling [28]. Exemplary lentiviral expression vectors comprising the TCR construct and reporting gene are provided in Figure 1.

Several cell lines are selected for testing TCR expression and activity, including Jurkat E6-1 (a control strain that expresses TCR), Jurkat J.RT3-T3.5 (a strain that lacks TCR β), SupT1 (a strain with damaged TCR α), and primary human T cells (which contain a diverse population of TCR clonotypes).

Jurkat E6-1 cells are an established human T lymphocyte cell line from peripheral blood. It is used as a control cell line expressing TCR [15, 18].

Jurkat RT3-T3.5 lacks TCR β due to a mutation that precludes expression of the TCR β -chain. It also fails to express surface CD3 or produce the T-cell receptor α,β heterodimer. It is therefore used for validating T-cell receptor gene transfer [1, 6, 21, 49].

SupT1 is a human lymphoblast line expressing multiple T lineage markers and is used because it encodes a non-functional receptor and fails to express TCR α [49].

PMBC-derived primary human T cells contain a diverse repertoire of TCR clonotypes.

Lentiviral vectors containing the antigen-specific TCR as illustrated in Figure 1 are used to transduce cancer cell lines lacking at least one TCR chain, and are subsequently assessed for expression using fluorescence-activated cell sorting (FACS). In cancer cell lines containing the antigen-specific TCR, luciferase is added to the cell line following successful stable expression to enable its use in mouse studies.

TCR constructs for mRNA, multicistronic mRNA, and lentiviral transduction proceed straightforwardly, via a screen based on the genetically encoded eGFP and labeling with anti-TCR or anti-CD3 antibody [3, 19].

Example 2: Construction of MHC-Based Chimeric Receptors (MHC-CAR) and T-cells Expressing Such

(i) Design of MHC-CAR Constructs

Receptors for adoptive cell therapy that genetically link the MBP 84-102 epitope to human leukocyte antigen HLADR2 are generated and, either incorporate or lack chimeric intracellular signaling domains [29]. The antigen-major histocompatibility complex (Ag-MHC) domain serves as receptor, binding the TCR of MBP-specific target cells. The Ag-MHC-CAR has been validated in preclinical mouse models with CD3- ζ (i.e. a first-generation signaling domain), which may optionally be in combination with additional co-stimulatory signaling domains (i.e. second- or third-generation signaling domains) for efficacy in humans, following the methodology provided in [9, 25]. A schematic illustration of the various designs of MHC-CAR is provided in Figure 2.

MHC-CARs are designed based on the structure of HLA-DR, and combined with a variety of internal cytoplasmic costimulatory domains. The MHC-CAR has two subunits: (i) an α -chain that contains the leader sequence, DRA*1010 domain, and a cytoplasmic domain; and (ii) a β -chain that contains a leader sequence (from HLA-DRB1*1501), a peptide (DENPVVHFFKNIVTPRTPP (SEQ ID NO: 15) from myelin basic protein), a domain (from HLA-DRB1*1501), and a cytoplasmic signal domain, for example, CD3 ζ [29]. Figure 3. The DNA used to create mRNA contains either single chains (shown as one embodiment in Figure 4) or are multicistronic and separated by orthogonal 2A sequences as illustrated in Figure 5. Genetically encoded eBFP or RQR8 are introduced into the cells for cell-labeling or to provide a mechanism for depletion [4, 5, 37, 40]. Figures 3 and 5.

As illustrated in Figure 6, the designed MHC-CAR has a number of sites for tagging. Site 1 is an HLA-DR antibody binding site for cases where native HLA-DR is either not expressed due to CIITA editing [26, 38]. Sites 2 and 3 are potential insertion sites for polyhistidine-tag motifs [24]. Sites 4 and 5 represent RQR and RQR8, respectively [37]. Multiple sclerosis is shown to affect blood brain barrier permeability, so in order to enhance the safety of therapeutic MHC-CAR, genetically encoded RQR would allow for rapid depletion of MHC-CAR T cells upon administration of rituximab, a chimeric monoclonal antibody treatment used offlabel in the treatment of severe MS [42].

(ii) Construction of T Cell Lines Expressing MHC-CAR

A number of cell lines discussed below are selected for testing the MHC-CAR construct. Assays are then continued in human T cells in order to establish clinically

translatable protocols.

K56 cells lack Class I and Class II MHC, allowing for tagless verification of HLA-DR expression using antibodies (HLA-DR is a component of the MHC-CAR) [45]. Expression assays will allow assessment of the MHC-CAR expression relative to RQR8 expression using flow cytometry, and the same lentiviral construct is then used in PMBC-derived T cells [23].

KM-H2 is a human Hodgkin's lymphoma line that can be used as an HLA-DR positive control line [34, 44]. Jurkat E6-1, as noted above, conditionally express HLA-DR upon delivery of CIITA and can be used to evaluate CIITA TALEN if CIITA knockout is used [33].

In addition, primary human T cells can be used. PMBC-derived primary human T cells are purified and enriched from whole blood, and then activated. Transduction follows enrichment. The cells are incubated with recombinant human interleukin-2 (and/or IL-7 and/or IL-15) [7, 12, 43]. Based on preclinical studies and the anticipated therapeutic course, the desired cell type is CD8⁺ T cells with the molecular and functional features of stem cell memory TSCM, central memory TCM, and naive cells TN [11, 22, 32, 35]. Antibody staining allows for cell immunotyping [27].

Initial constructs, including a permutation of signaling domains (CD3z, 41BB, CD28) are expressed using mRNA, multicistronic mRNA, and lentiviral strategies in K562 cells. The cell line's lack of MHC Class I and II allow the tagless verification of MHC-CAR expression using the HLA-DR antibodies, following a successfully employed strategy [29]. Quantification of the expression efficiency in more clinically relevant cell lines, including therapeutic T cells, depends on construct, and will be measured using genetically encoded fluorescent reporters (BFP) or antibody staining of the RQR site, a polyhistidine- (HIS)-tag, or the MHCCAR (with an HLA-DR antibody if CIITA is inactivated using TALEN). Labeling sites are indicated in Figure 6. To test rates of MHC-CAR delivery as a potential therapeutic vector, BFP is removed from constructs while RQR is retained to provide for depletion control. Expression rates of the clinically relevant construct are measured using antibody staining post-editing.

The transcription activator-like effector technology (TALEN) can also be used for preparing T cells expressing MHC-CAR. Human T cell lines are activated, transfected with TALEN, and either transfected or transduced with MHC-CAR. They are then stained and analyzed by flow cytometry to assess TALEN gene inactivation and MHC-CAR expression.

Upon verification of construct expression in desired cell lines, TALEN transduction (TCRa and CD52 or CIITA as discussed below) into human T cell lines is performed. TALEN is introduced into activated human T cell lines, and the MHC-CAR is subsequently introduced into the same T cell lines for evaluation. The modification of the human T cells can be performed in this order to prevent incidental fratricidal killing of MHC modified cells due to the native TCR [10]. The activity of TALEN-edited MHC-CAR T-cells is to be confirmed.

(iii) Modification of MHC-CAR-Expressing T Cells via Transcription activator-like effectors (TALENs)

Human T cells are selected to confirm inactivation of TCRa, and CD52 or CIITA genes by TALEN, followed by evaluation in combination with MHC-CAR, using TALENs. Transcription activator-like effectors (TALENs) bind DNA in a sequence-specific manner. The DNA binding domain contains a highly conserved 33-34 amino acid sequence with a divergent 2 amino acid RVD (repeat variable dipeptide motif) conferring specific nucleotide recognition [23, 31].

Mutation of either α or β chain of the TCR is sufficient for disruption of surface TCR expression [15, 36]. TALEN is used to inhibit expression of two genes in the therapeutic cell without introducing a proliferative advantage through undesired translocations [39][47]. TCR expression is inhibited through a TRAC-targeted TALEN to prevent graft versus host disease (GvHD) and allow for the creation of an allogeneic therapy [15, 39, 47].

CD52 deletion can be made for alemtuzumab compatibility. Alemtuzumab is a humanized anti-CD52 IgG1 monoclonal antibody that targets and depletes circulating T and B lymphocytes [41]. Alemtuzumab can be used as rescue therapy or as first line drug in severe-onset MS [50], and will be co-administered in the human patient population. TALEN knockout will make MHC-CAR compatible with concurrent treatment in patients [14, 39]. Further, CIITA deletion can be made for characterization of HLA-DR in MHC-CAR. CIITA is a protein coding gene essential for the transcriptional activity of HLA Class II promoter [26]. Knockout would allow for the direct measurement of HLA-DR using antibodies for characterization of MHC-CAR expression [38, 53]. The inhibition of CD52 expression allows for concurrent treatment with Lemtrada R (alemtuzumab), an FDA-approved treatment for multiple sclerosis [42]. Alemtuzumab is also used as a lympho-depleting/lympho-suppressive agent that aids in the engraftment of CAR T therapies [39]. A CAR T-cell therapy modified with TRAC- and CD52-targeted TALENs is currently being

tested in clinical trials [38, 40, 43].

TALEN to inactivate MHC Class II transactivator (CIITA) in place of CD52, as inactivation of CIITA is expected to inhibit HLA-DR expression [26][38], thus allowing for the direct identification of MHC-CAR-containing cells by HLA-DR antibody staining.

Validated TALENs (TRAC:

TTGTCCCACAGATATCCagaacctgacctgCCGTGTACCAGCTGAGA (SEQ ID NO: 376), CD52: TTCCTCCTACTCACCATcagcctcctggttatGGTACAGGTAAGAGCAA (SEQ ID NO: 371), CIITA:

TTCCCTCCCAGGCAGCTCacagtgtgccaccaTGGAGTTGGGGCCCCCTA (SEQ ID NO: 366)) are obtained from Collectis or designed to target previously validated sites [38, 43].

Human T cells are activated and electroporated with mRNA encoding variants of 3 different TALEN: TCR-alpha constant chain, CD52, and CIITA. Cells are surface stained with anti-CD3 or anti-TCR (TCR-alpha constant chain), anti-CD52 (CD52), or anti-HLA-DR (CIITA), and then analyzed by flow cytometry.

In the TALEN expression assays, the ability of previously validated TALENs to inactivate target genes are re-validated [39]. Translocation studies and off-target studies are reperformed, and several whole-genome sequences confirm results. GUIDE-seq can be used as an alternative to whole genome sequencing to confirm on- and off-target editing [8].

One of more of the following endogenous genes are to be edited to reduce interaction with other cells: TCR (through TCR alpha or beta chain; to reduce targeting to undesired cells), CIITA (regulates expression of MHC Class II genes; target cells for faster deletion), B7-1(CD80) and/or B7-2(CD86) knockout, and b2m (regulates expression of MHC Class I genes) with NKG2D ligands or UL18.

Further, one or more of the following genes can be edited to modify the function of an interacting cell: PD-L1 +/- CTLA4-Ig overexpression; PD-L1/2 overexpression +/- PD-1 knockout; FasL overexpression +/- Fas knockout; Galectin 9 overexpression +/- TIM3 knockout; and/or PVT/CD155 overexpression +/- TIGIT knockout. Defects in PD-1, Fas, Tim3, TIGIT predispose patients to autoimmunity. Some drugs can restore function (for example: Tim3, glatiramer acetate and IFN-beta). If autologous cells from a patient suffering autoimmune disease are utilized they may require patient specific correction of defective genes that influence CTL and Treg function. Their personal mutation set may also determine whether CTL or Treg cells will be the most therapeutically relevant, and whether some cellular modification will be effective, if either autologous or allogeneic cells are used.

Further, the following edits may modify the location and/or function of the cells (for example, to make it more like a follicular regulatory cell):

- MHC-CAR-(FOX3P)-(CS1 or CD19 CAR)/(CS-1 knockout required for CS-1 CAR)
- MHC-CAR-(FOX3P)-(CCR7 or CXCR5)

Moreover, the following edits can enable combination therapies for autoimmune diseases (*e.g.*, MS specific therapeutics)

- RQR tag: engineered T cells can be removed with rituximab (kill switch). A tag for the newly approved anti-CD20 antibody Ocrevus could be generated.
- Rapamycin switch : CARs only in/active when patient is treated with rapamycin (tacrolimus)
- CD52 knockout: allows pretreatment with Lemtrada (alemtuzumab) to decrease number of immune cells present
- VLA-4 knockout: can treat with tysabri to move pathogenic immune cells to periphery but engineered cells are forced to stay there (may not be ideal for patients with established MS as cells that are stuck in the brain spinal cord; however, simultaneous mRNA expression of VLA-4 can provide temporary access to those locations)
- IL-6 antibody (Tocilizumab) secretion from engineered T cell: helpful when the engineered T cell must access the brain and spinal cord, but this drug cannot access the locations due to the BBB

Example 3: Investigation of MHC-CAR Activities

(i) Preparation of primary T cells expressing MHC-CAR

Primary T cells can be prepared as follows. T cells are isolated from peripheral blood mononuclear cells (EasySep Human T Cell Enrichment Kit, Stemcell Technologies) and activated (Dynabeads Human T-Activator CD3/CD28, Life Technologies) with (X-Vivo 15 medium, Lonza; 20 mg/ml IL-2, Miltenyi; 5% human AB serum, Seralab). A suitable MHC-CAR construct containing a MBP antigenic peptide is introduced into the primary T cells using a conventional method. Surface expression of the MHC-CAR construct is verified by FACS and antibody staining.

(ii) MHC-CAR activity tests in vitro

Upon verification of construct trafficking and expression (with mRNA, multicistronic mRNA, lentivirus), activity tests are conducted in vitro. All tests are conducted at different effector:target (E:F) cell concentrations. The in vitro tests provide an initial evaluation of

MHC-CAR signaling domains and T cell subsets.

(iii) *Signaling domain assessment by IL-2 production*

24 hours post electroporation, human T cells transiently engineered with MHC-CAR are stimulated with plate-bound HLA-DR antibody, to determine whether MHC-CAR (containing various signaling domains) is functional. IL-2 production is measured 24 hours later using a StemCell IL-2 ELISA kit. This test provides a quick assay as to whether variants should be reengineered or abandoned [29].

(iv) *Interaction of MHC-CAR cells and pathogenic TCR cells through proliferation assay*

Target cell lines transiently expressing TCR are magnetically sorted for TCR expression 24 hours after electroporation and irradiated, in order to test whether engagement of the MHC-CAR with antigen-specific TCR stimulates proliferation of MHC-CAR containing T cells. Alternatively, target cell lines that stably express TCR are irradiated. The irradiated cells displaying (+/-antigen-specific) TCR are incubated with CFSE-labeled MHCCAR cells and proliferation is measured after culture at different T:E ratios [30].

(v) *Degranulation assay*

CAR T-cells are labeled through epitopes on RQR (which are not being expressed on the target cells used) or eBFP instead of T cell markers. The assay is performed for cell lines with transient or stable expression (the example of transient expression is described). 24 hours post-electroporation, MHC-CAR human T-cells with either RQR8 or BFP are co-cultured with target (antigen-specific TCR SupT1 or Jurkat) or control (+/- TCR Jurkat or TCR- SupT1) cells for 6 hours. Transiently expressed (and later stably expressing) target cells are electroporated with antigen-specific TCR and sorted with CD3 magnetic beads post-electroporation. The RQR+ or BFP+ MHC-CAR T-cells (as identified with anti-rituximab antibody, QBEnd10 antibody) are analyzed by flow cytometry to detect the expression of the degranulation marker CD107a on their surface [2].

(vi) *Cytokine secretion assay*

The assay is performed for cell lines with transient or stable expression (transient expression is described). The human T cells transiently expressing the MHC-CARs are assessed for cytokine secretion following co-culture with target cells 24 hours post

electroporation. Human T cells transiently expressing the MHC-CARs are co-cultured with target (antigen-specific TCR containing Jurkat or SupT1 cells) or control (+/- non-antigen-specific Jurkat or SupT1) cells for 24 hours. The antigen-specific TCR is then killed by irradiation before the assay. The supernatants are harvested and analyzed using the TH1/TH2 cytokine cytometric bead array kit to quantify the cytokines produced by T cells [13]. MHC-CAR T-cells produce IFN and other cytokines in co-culture with antigen-specific TCR expressing target cells but not in co-culture with control cells.

(vii) *IFN γ release assay*

Various levels of MHC-CAR expressing cells are incubated with irradiated TCR T-cells 24 hours after transfection. Co-cultures are maintained for 24 hours. After incubation and centrifugation, supernatants are tested with IFN γ detection by ELISA.

(viii) *Cytotoxicity assay*

TCR T-cells are incubated with therapeutic MHC-CAR as well as control cells. Target and control cells are labeled with fluorescent intracellular dyes (CFSE or Cell Trace Violet) before co-culturing with MHC-CAR T-cells. The co-cultures are incubated for 4 hours. After this incubation period, cells are labeled with a fixable viability dye and analyzed by flow cytometry. Viability of each cell population (target or negative control) is determined and the percentage of specific cell lysis is calculated. Cytotoxicity assays are carried out 48 hours after transduction.

(ix) *Inhibition Assay*

PBMCs are co-cultured with irradiated or mitomycin-treated engineered T cells expressing the MHC-CAR construct. As control, PBMCs are co-cultured with irradiated or mitomycin-treated engineered T cells that do not express the MHC-CAR construct. 7 days later, cell proliferation from a human patient donor A is measured by XTT colorimetric assay or by CFSE dilution (FACS analysis). Although cell proliferation would be observed in control, no or limited cell proliferation is expected when engineered T cells express secreted FP. The results from this experiment aim to show that alloreactive T cells proliferation is inhibited when the MHC-CAR expressing T cells express FP.

(x) *Proliferation*

To test whether engagement of the MHC-CAR with antigen-specific TCR stimulates proliferation of MHC-CAR containing T cells, target cell lines transiently expressing TCR

are magnetically sorted for TCR expression 24 hours after electroporation and irradiated. Alternatively, target cell lines that stably express TCR are irradiated. The irradiated cells displaying (+/-antigen-specific) TCR are incubated with CFSE-labeled MHC-CAR cells and proliferation is measured after culture at different T:E ratios.

(xi) *In Vivo Tests*

MHC-CAR (with mouse and human MHC) in mouse T cells has previously shown therapeutic efficacy in experimental allergic encephalomyelitis, the mouse model for multiple sclerosis [25, 29]. Here we test whether MHC-CAR in human cells can target human TCR in human T cell lines, using an *in vivo* mouse model analogous to that in prior CAR T preclinical studies [39]. The *in vivo* tests allow further evaluation of MHC-CAR signaling domains and T cell subsets.

The *In vivo* activity of MHC-CAR T-cells can be verified in a mouse xenograft model as illustrated in Figure 7.

Immunodeficient NOG mice are intravenously injected with antigen-specific TCR-luciferase expressing T-cells as an MS xenograft mouse model. Mice then receive intravenous doses of MHC-CAR T-cells tested at different doses, either 2 or 7 days post-injection with tumor cell line. Intravenous injection with T-cells that are not transduced with the CAR lentiviral vector serve as control. Bioluminescent signals are determined at the day of T-cell injection (D0), at D7, 14, 21, 28 and 40 after T-cell injection in order to follow the expansion of TCR-luciferase expressing cells in different animals [39].

CAR T-cells with similar background modifications (TALEN to inactivate native TCR and minimize graft vs host, TALEN to inactivate CD52 and allow simultaneous treatment with alemtuzumab, and RQR8 to allow depletion) to the ones shown here have been previously validated in mouse models and are now used in a UCART19 clinical trial [39].

(xii) *Kill-switch verification*

Transduced T cells are exposed to 25% baby-rabbit complement (AbD Serotec) for 4 hours with or without inclusion of pharmaceutical complements (rituximab, tysabri, or alemtuzumab) to examine complement-dependent cytotoxicity (CDC)-mediated sensitivity. Miltenyi CD34 magnetic bead--selected-transduced RQR8 T-cells are compared against a similarly treated population of Q8-transduced T cells to demonstrate specificity of CDC-

mediated deletion. Further examination of CDC assay parameters was achieved through time-course/dose-titration assays using RQR8-transduced T cells incubated with pharmaceutical complement at 12.5, 25, 50, and 100 mg/mL and time-point assessments ranging between 1 to 120 minutes.

Example 4: Regulatory T Cells (Treg) Expressing MHC-CAR

Therapies that antigen specifically target pathologic T lymphocytes responsible for multiple sclerosis and other autoimmune diseases are expected to have improved therapeutic indices compared with antigen-nonspecific therapies. This example provides an exemplary cellular immunotherapy that uses chimeric antigen receptors to selectively redirect therapeutic T cells against myelin basic protein-specific T lymphocytes implicated in MS

Treg Cell Sorting, Transduction, and Expansion

CD4⁺ T cells are isolated from PMBC via RosetteSep (STEMCELL Technologies) and enriched for CD25⁺ cells (Miltenyi Biotec) prior to sorting into live CD4⁺CD45RO⁻CD45RA⁺CD25⁺ Tregs and CD4⁺CD45RO⁻CD45RA⁺CD25⁻ control T cells by FACS. Sorted T cells are stimulated with artificial APCs (aAPCs) loaded with α CD3 mAbs in 1,000 U/ml or 100 U/ml of IL-2, for Tregs or non-reg control T, respectively. One day later, cells are transduced with lentivirus. At day 7, Δ NGFR⁺ cells were purified with magnetic selection (Miltenyi Biotec), then re-stimulated with aAPCs as above and expanded for 6 to 7 days.

Flow Cytometry:

For phenotypic analysis, cells were stained with fixable viability dye (FVD) (65-0865-14 and 65-0866-14, eBioscience) and for surface markers before fix/perm with FOXP3/Transcription Factor Staining Buffer Set (eBioscience), followed by staining for intracellular proteins. For analysis of cytokine production, cells were stimulated with 10 ng/ml PMA and 500 ng/ml ionomycin, in the presence of brefeldin A (10 μ g/ml) (all Sigma-Aldrich) for 4 hours. Samples were analyzed by flow cytometry.

Microscopy:

PBMCs are labeled with PKH26 or PKH67 (Sigma-Aldrich, PKH26GL-1KT and PKH67GL-1KT), and Tregs are labeled with cell proliferation dye (CPD) eFluor450 (eBiosciences, 65-0842-85) and then suspended in a 3D gel of 1.5% rat tail collagen type I

(Ibidi) composed of 1× DMEM and 10% FCS per the manufacturer's general 3D gel protocol. The cell suspension is pipetted into a μ -Slide Chemotaxis3D and allowed to polymerize for 30 minutes in a humidified incubator at 35°C and 5% CO₂ (Tokai Hit) on a Leica TCS SP8 confocal microscope. The outer chambers are filled with 1× DMEM and images recorded using a $\times 10/0.30$ objective every 2 minutes for 3 hours. eFluor450, PKH67, and PKH26 were excited at 405 nm, 488 nm, and 561 nm, and the fluorescence emission is collected at 415–470 nm, 495–525 nm, and 570–650 nm, respectively. The number of interactions between CAR-Tregs and either target or control cells is quantified every 20 minutes. Cells that do not move were excluded from the analysis. The total numbers of each labeled cell type per field of view can be counted using the analyze particles function in ImageJ (imagej.nih.gov/ij/).

Treg-Specific Demethylated Region (TSDR) analysis

Treg stable expression of stable Foxp3 is associated with selective demethylation of TSDR within the Foxp3 locus. In order to test for stable expression, DNA from frozen T cell pellets was isolated with the DNeasy Blood and Tissue Kit (QIAGEN) and bisulfite converted with the EZ Direct Kit (Zymo Research). PCR of BisDNA was performed with the Human FOXP3 Kit (Epigen DX) and prepared for pyrosequencing using PyroMark buffers (QIAGEN), then assayed on a Biotage PyroMark Q96 MD pyrosequencer (QIAGEN). Results were calculated with Pyro Q-CpG software (Biotage).

Cytokine production

To measure cytokine production, T cell lines are stimulated with K562 cells (1 K562:2 T cells) for 48 hours. Supernatants are collected and cytokine concentration was determined by the Human Th1/Th2/Th17 Cytokine Kit (BD Biosciences) and analyzed.

Suppression of MHC CAR-specific proliferation

To test whether Treg specific for target were also capable of suppressing CD4⁺ T cell proliferation, MHC CAR-specific CD4⁺ T clones are isolated. An Epstein Barr Virus-transformed B lymphoblastoid cell line was transduced with MHC-CAR using lentivirus. EBV cell lines were grown overnight, irradiated at 150 Gy, and cocultured with CPD-labeled MHC CAR-specific CD4⁺ T clones in the absence or presence of CAR-expressing Tregs or conventional T cells. Proliferation is determined after 4 days, and percentage of suppression

of MHC CAR-specific clones calculated using percentage of proliferation as follows: $(100 - [(\% \text{ proliferated MHC CAR} + \text{test})/(\% \text{ proliferated MHC CAR alone})] \times 100)$.

Upon verification of construct trafficking and expression (with mRNA, multicistronic mRNA, and/or lentivirus), activity tests will begin *in vitro*. All tests are conducted at different effector:target (E:F) cell concentrations. The *in vitro* tests are expected to provide an initial evaluation of MHC-CAR signaling domains and T cell subsets.

Transient or lentiviral expression of chemokine and adhesion receptors in T cells

Receptors are expressed in human T cells after electroporation of mono/polycistronic mRNA or lentiviral transduction. Expression of the receptor is analyzed using flow cytometry. In summary: 5×10^6 T cells preactivated several days (3-5) with anti CD3/CD28 coated beads and IL2 were re-suspended in cytoporation buffer T, and electroporated with 45 μ g of mRNA. Twenty-four hours after electroporation, human T cells engineered using polycistronic mRNAs encoding the multi-chain CARs were labeled with a fixable viability dye eFluor-780 and a PE-conjugated goat anti mouse IgG F(ab')₂ fragment specific, and analyzed by flow cytometry. Alternately the receptors were vectorized in lentivirus, expressed, and analyzed similarly.

In vitro chemotaxis assay

Transduced T cells were used in chemotaxis assays as previously described [Bürkle et al., *Blood*, 110(9):3316-3325, 2007; Wu and Hwang, *Journal of immunology*, 168(10):5096, 2002.; Singh et al., *Journal of immunology*, 180(1):214-221, 2008.; Ryu et al., *Molecules and Cells*, 39.12:898-908, 2016.]. Cells (~20,000 cells in medium, one million cells, 5×10^6 /mL) were placed on top of the 5- μ m pore size filters in duplicate, whereas medium with and without chemokines were placed into the lower chamber. After 30 min, 1 h, 3 h, 5 hr, 24 hr at 37°C, migrated cells that had fallen to the bottom of the plate were:

A. photographed using a 4 $\times$ objective. Three random views from each of two wells were counted using Image Pro Plus (Media Cybernetics, Silver Spring, MD). Three independent experiments were performed with similar results.

B. 400 μ L of the cell suspension was added to 100 μ L of a solution containing 4×10^{-7} M FITC-labeled phalloidin, 0.5 mg/mL 1-alpha-lysophosphatidylcholine (both from Sigma, St Louis, MO), and 7% formaldehyde in phosphate-buffered saline (PBS). The fixed

cells were analyzed by flow cytometry on a FACSCalibur, and all time points are plotted relative to the mean relative fluorescence of the sample before addition of the chemokine.

C. the cells in the lower chamber were counted using Countess II FL (Thermo Fisher Scientific, USA) or the O.D. value at 450 nm was measured using a Versamax microplate reader (Molecular Devices).

Example 5: Expression of MHC-based Chimeric Receptors (MHC-CAR) in HEK293 cells

Constructs encoding MHC-CARs were constructed as discussed in Example 2 and assessed for expression in HEK293 cells. Briefly, Construct 1 includes an EF1alpha short promoter, CD19 CAR (4G7-CAR), CCR6, and GFP (provided by SEQ ID NO: 426); and Construct 2 includes a EF1alpha short promoter, RQR8, MHC-CAR1 part B MHC-CAR1 part A, and GFP (provided by SEQ ID NO: 409).

Constructs 1, 2, or media control (non-transfected) was transfected into HEK293 cells and cultured. The cells were assessed for expression by microscopy based on GFP expression. Populations of GF-positive cells were observed in the groups that were transfected with Construct 1 or Construct 2. The cells were also assessed for expression of the components encoded by the constructs by flow cytometry. Tables 8 and 9.

Construct 1 Expression

For detection of CCR6, cells were incubated with an anti-CCR6 monoclonal antibody conjugated to APC (17-1969-42, eBioscience); and for detection of CD19, cells were incubated with a biotinylated CD19 (Acro CD9 – H8259, Acro Biosystems) followed by a streptavidin-PE (405203, BioLegend®).

Table 8: Expression of Construct 1

CCR6 Expression	FITC (GFP)	APC (CCR6)	PE (CD19)
Unstained	71.9	0.1	17.4
CCR6	71.3	81.9	16.9
CD19 Expression	FITC (GFP)	APC (CCR6)	PE (CD19)
Unstained	71.9	0.1	17.4
CD19	71.3	0.1	33.3
streptavidin-PE only	71.5	0.1	18.5

Construct 2 Expression

For detection of CD34 expression from the epitope included in RQR8, cells were incubated with an anti-CD34 APC-conjugated monoclonal antibody; and for detection of MHC-CAR expression, the cells were incubated an anti-HLA-DR antibody.

Table 9: Construct 2 Expression

GFP Expression	FITC (GFP)	APC (CD34)	PE
Unstained	62.3	0.1	2.4
RQR Expression	FITC (GFP)	APC (CD34)	PE
Unstained	62.3	0.0	2.4
CD34	61.8	58.0	2.5
MHC CAR Expression	FITC (GFP)	APC (HLA-DR)	PE
Unstained	62.3	0.0	2.4
HLA-DR	63.9	98.1	2.1

Nucleic acid sequence of Construct 1 (SEQ ID NO: 426)

atggagacagacactcttctccttttgggtcttctgctgctgtgggttcccggaagcacaggagaagtacagttgcaa
cagtctggggccagaactcatcaaaaccggagcttctgtaaaaatgtcatgcaaagctagtggaatatacatttact
tcttacgtgatgcaactgggtaaaaacagaaacctgggtcaggggcttgagtggaatcgggtacattaacccatataat
gaaggcaccataataacagagaaattcaagggaagggtacgcttacatcagataagtcagtagcaccgcttat
atggaacttagcagccttacttccgaagattccgcggtgtattactgcgcgagaggacttactactacgggagt
cgagtattcgattattgggtcaaggcacgacgctcacggtgagctcaggtggaggagggtctgggggtggcggc
agtgggtggggggggctcagacatcgtgatgacccaggcagcacccttctatcccggtaccccgaggcgagtctgta
tctatcagttgtcgggtccagcaagttcttctcaacagtaattggcaatacatatcttactggttccctccaaagg
cctgggcaaagtcctcaactcttcttatatcggatgtccaatcttgcgagtggcgtacccgacaggttttcaggg
tctgggagcggaacagcttttacggtgagaatatccagggtagaagctgaggacgtcggtgtatattattgcatg
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ccagcgccacgaccacctaagcccgctccaaactattgctcccgagccctgagttctcgccagaagcgtgtaga
cctgctcccgcggggcttccatagcggggccttgactttgcatgtgatattctatatatgggctcctttggcg
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gaggaggagggggggtgtgagcttaggggtcaagtttctcgtcttgccgacgcgccagcctatcaacaggggcaa
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attgcgctgggtgttggcttcttggcctgtcagatcccccataatatgggtctcgtcgtcaacggcgctaacctg

ggtaagatgaatcgatcttgtcagtcgagaaagttgatcggtacacacaaaactgtgacagaagtgctggccttc
cttcactgttgctgaacccagttttgtatgcttttataggacagaagtttcgaaattacttcttgaaaatcctc
aaggacctctggtgtgttcgaaggaagtacaagagctctggcctttagttgcgctggcgctacagtgagaatata
tccccgcagacctccgagactgctgataatgacaacgcaagttccttcactatg (SEQ ID NO: 426)

Nucleic acid sequence of Construct 2 (SEQ ID NO: 409)

atgggtacttcactgttgctggtggcactttgtcttttgggtgccgatcatgctgatgcatgtccgtactcc
aatcctagcctgtgctccgggggggagggagtgaactccctacacagggaaccttctctaattgtctccaccaac
gtctccctgcaaaacgaccacacagcttgccctatagtaaaccttccctctgtagtggaggggggggttca
cctgctccacgacctctacccccggcccaacgatcgctgcacaaacgctcagttcttaggcgggaagcctgtagg
ccagcggctggcgggtgcgggttcatacgcggggattggattttgcctgcgacatttacatttgggctccgctggcc
ggtaactgtgggggtattgctgttgctctcttggattttacgctttattgcaatcacaggaaacaggcgacgagtgc
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ggcgacacctgacgggagtaactggaactcccaaaaggatattttggagcaggcacgagcagctgtggacacctat
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accttccaaactttggtaatgttggaacgggtgcccgcgacccggggaggtgtatacatgccaagttgaacacccg
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aagttttcccgaaagtgcggacgctcccgcgatcagcaagggtcaaaaacagctttacaacgaactgaacttgga
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ggtgaacggaggagaggaagggacatgacggactttatcaaggattgtctaccgcaactaaagacacctatgac
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ctcacaattctcccgtagaacttagggaaacctaacgtcctcatatgtttcatcgacaagttcactcctccggtg
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atcctggctgagctggacggcgacgtaaacggccacaagttcagcgtgtctggcgaggcgaggcgatgccacc
tacggcaagctgacctgaagttcatctgcaccacccggcaagctgcccgtgcccgtggcccaacctcgtgaccacc
ctgacctacggcgtgcagtgcttcagccgctaccccgacacatgaagcagcacgacttcttcaagtcggccatg
cccgaggctacgtccaggagcgacacctcttcttcaaggacgacggcaactacaagacccgcgcgagggtgaag
ttcgaggggcgacacctgggtgaacgcgcatcgagctgaagggtcgaacttcaaggaggacggcaacatcctgggg
cacaagctggagtacaactacaacagccacaacgtctatatcatggccgacaagcagaagaacggcatcaaggcg

aacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgccgaccactaccagcagaacacccccatc
 ggcgacggcccccggtgctgctgctgcccgaacaaccactacctgagcaccacagtcggccctgagcaaaagaccccaacgag
 aagcgcgatcacatggctcctgctggagttcgtgaccgcgcgcccgggatcactctcggcacgagcgtgtacaag
 taa` (SEQ ID NO: 409)

Example 6: Expression of CD3

Expression of CD3 was assessed to determine the efficacy of CRISPR methods targeting the T cell receptor alpha constant (TRAC) gene. Briefly, sgRNAs targeting the TRAC gene were generated by amplifying the target site using forward primer (5'-AGCGCTCTCGTACAGAGTTGG-3' (SEQ ID NO: 385)) and reverse primer (5'-AAAAAAGCACCGACTCGGTGCC-3' (SEQ ID NO: 386)).

The unmodified sgRNA is provided by the nucleic acid sequence:

5'- GAG AAU CAA AAU CGG UGA AUG UUU UAG AGC UAG AAA UAG CAA GUU AAA
 AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA CCG AGU CGG UGC UUU
 U -3'. (SEQ ID NO: 384)

The modified sgRNA is provided by the nucleic acid sequence:

5'- 2'OMe(G(ps)A(ps)G(ps)) AAU CAA AAU CGG UGA AUG UUU UAG AGC UAG
 AAA UAG CAA GUU AAA AUA AGG CUA GUC CGU UAU CAA CUU GAA AAA GUG GCA
 CCG AGU CGG UGC 2'OMe(U(ps)U(ps)U(ps) U -3' (SEQ ID NO: 387).
 2'OMe=2'O)-methyl RNA and ps=phosphorothioate.

Primary human stimulated CD3+ T-cells were transfected with an mRNA encoding Cas9 (Cas9 only) or both an mRNA encoding Cas9 and sgRNAs targeting the TRAC gene. After 7 days post-transfection, expression of CD3 was assessed by flow cytometry. The cells were incubated with a 1:100 dilution of an anti-CD3-APC antibody (clone OKT3; BioLegend® cat. no. 317318). As shown in Figure 11, transfection of an mRNA encoding Cas9 and sgRNAs targeting the TRAC gene resulted in a substantial reduction in CD3 expression.

Example 7: Kill switch verification

Efficacy of the RQR8 kill switch encoded in example Construct 2 was assessed using a cell viability assay. Briefly, HEK cells were transfected with media only, Construct 1 (SEQ ID NO: X), Construct 2 (which encodes the rituximab-mediated RQR8 kill switch, SEQ ID NO: X), or both Construct 1 and Construct 2. The transfected HEK293 cells were harvested, counted, and resuspended at 1×10^6 cells/mL. 300 μ L of the cellular suspension was

transferred into each of 4 wells of a 48-well tissue culture plate. 100 uL of complete medium and 4 uL of Rituximab were added to the second well, and 100 uL of freshly prepared baby rabbit complement and 4 uL of Rituximab were added to the fourth well. The plates were incubated for 2, 4, or 24 hours. The assay was terminated by the adding 1 uL of chilled Annexin V buffer (150 mMNaCl, 10 mM HEPES, 10 mM CaCl), and then the sample was transferred into a pre-prepared flow cytometry tube containing 3 mL of Annexin V buffer.

Samples were harvested by centrifugation and any residual buffer was blotted with paper towels. The samples were then stained with 1 uL of Annexin V APC, vortexed, and placed in subdued lighting for 15 minutes. The samples were then washed with Annexin V buffer and supplemented with 5 uL of propidium iodide/mL buffer and placed on ice pending flow cytometry performed immediately following final suspension.

The percentage of GFP-positive cells was quantified to quantify the killing induced by the RQR8 kill switch. As shown in Figure 12, incubation with complement alone resulted in some cell death, however this reduction in viability was observed in all of the groups of cells. Cell death was observed in cells that expressed Construct 2 or both Constructs 1 and 2 the presence of the combination of rituximab and complement, indicating specific RQR8-mediated cell death.

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OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

What Is Claimed Is:

1. A major histocompatibility complex (MHC)-based chimeric receptor, comprising:
 - (i) an extracellular domain of a MHC molecule conjugated to an antigenic peptide from an antigen involved in an autoimmune disease; and
 - (ii) a cytoplasmic signaling domain, at least one co-stimulatory domain, or a combination thereof.
2. The MHC-based chimeric receptor of claim 1, which comprises the at least one co-stimulatory domain.
3. The MHC-based chimeric receptor of claim 2, wherein the at least one co-stimulatory domain is a co-stimulatory domain from 4-1BB (CD137), a co-stimulatory domain from CD28, or a combination thereof.
4. The MHC-based chimeric receptor of claim 2 or claim 3, wherein the MHC-based chimeric receptor is free of a cytoplasmic signaling domain.
5. The MHC-based chimeric receptor of any one of claims 1-4, which further comprises a hinge domain located between (i) and (ii).
6. The MHC-based chimeric receptor of any one of claims 1-5, which comprises a cytoplasmic signaling domain of CD3 ζ .
7. The MHC-based chimeric receptor of any one of claims 1-3, wherein the antigenic peptide is from myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP), insulin, glutamate decarboxylase, or described in Table 1.
8. The MHC-based chimeric receptor of any one of claims 1-7, wherein the MHC molecule is a class I MHC.
9. The MHC-based chimeric receptor of claim 8, wherein the class I MHC is a human class I MHC.

10. The MHC-based chimeric receptor of claim 8 or claim 9, wherein the extracellular domain of the chimeric receptor comprises an extracellular domain of the alpha chain of the class I MHC, which is fused to the antigenic peptide.

11. The MHC-based chimeric receptor of claim 10, wherein the chimeric receptor is a fusion polypeptide comprising (i) the extracellular domain of the class I MHC molecule, and (ii) the cytoplasmic domain, the at least one co-stimulatory domain, or the combination thereof.

12. The MHC-based chimeric receptor of claim 11, wherein the chimeric receptor is a fusion polypeptide, which comprises, from N-terminus to C-terminus, a signal peptide, a first peptide linker, the antigenic peptide, a second peptide linker, an extracellular domain of macroglobulin, a third peptide linker, the class I MHC molecule, a transmembrane domain, the at least one co-stimulatory domain, and CD3 ζ .

13. The MHC-based chimeric receptor of any one of claims 1-7, wherein the MHC molecule is a class II MHC.

14. The MHC-based chimeric receptor of claim 13, wherein the class II MHC is a human MHC II.

15. The MHC-based chimeric receptor of claim 13 or claim 14, wherein the chimeric receptor comprises a first polypeptide, which comprises an extracellular domain of a first MHC class II, and a second polypeptide, which comprises an extracellular domain of a beta chain of a second MHC class II, and wherein the antigenic peptide is fused to either the first polypeptide or the second polypeptide, and wherein either the first polypeptide or the second polypeptide further comprises the cytoplasmic signaling domain, the at least one co-stimulatory domain, or the combination thereof.

16. The MHC-based chimeric receptor of claim 13 or claim 14, wherein the chimeric receptor is a fusion polypeptide comprising (i) an extracellular domain of the alpha chain of a first MHC class II molecule, (ii) an extracellular domain of the beta chain of a

second MHC class II molecule, (iii) the antigenic peptide, and (iv) the cytoplasmic signaling domain, the at least one co-stimulatory domain, or the combination thereof.

17. The MHC-based chimeric receptor of any one of claims 13-16, wherein the first MHC class II is HLA-DRA*1010.

18. The MHC-based chimeric receptor of any one of claims 13-17, wherein the second MHC class II is HLA-DRB1*1501.

19. A nucleic acid or a nucleic acid set, which collectively encodes the MHC-based chimeric receptor of any one of claims 1-18.

20. The nucleic acid or nucleic acid set of claim 19, wherein the nucleic acid or nucleic acid set is located in a viral vector(s).

21. A genetically modified immune cell, which expresses a MHC-based chimeric receptor of any one of claims 1-18.

22. The genetically modified immune cell of claim 21, which is a T cell.

23. The genetically modified immune cell of claim 22, wherein the activity of the endogenous T cell receptor (TCR) is suppressed.

24. The genetically modified immune cell of claim 23, wherein the alpha chain of the endogenous TCR, the beta chain of the endogenous TCR, or both are mutated or deleted to disrupt surface expression of the endogenous TCR.

25. The genetically modified immune cell of any one of claims 22-24, wherein expression of the endogenous CD52 is disrupted.

26. The genetically modified immune cell of any one of claims 21-25, further expressing a suicide gene, a marker gene, or both.

27. The genetically modified immune cell of claim 26, wherein the suicide gene is RQR8.

28. The genetically modified immune cell of claim 26, wherein the marker gene is a fluorescent protein gene.

29. The genetically modified immune cell of any one of claims 21-28, wherein the immune cell is further modified for lymph node delivery and retention.

30. The genetically modified immune cell of claim 29, wherein the immune cell is further engineered to overexpress VAP-1, L-selectin, CCR7, or a combination thereof.

31. The genetically modified immune cell of claim 29 or claim 30, wherein the expression of endogenous sphingosine-1-phosphate receptor 1 is disrupted in the immune cell.

32. The genetically modified immune cell of any one of claims 21-28, wherein the immune cell is further modified to express one or more surface molecules for tertiary lymph node or ectopic lymph node delivery and retention.

33. The genetically modified immune cell of any one of claims 21-32, wherein the immune cell is modified with IL6ST knockout, IL6R knockout, or both.

34. The genetically modified immune cell of any one of claims 21-33, wherein the immune cell is further modified to express or overly express a chemokine receptor.

35. The genetically modified immune cell of claim 34, wherein the chemokine receptor is implicated in migration of pathologic cells to an inflamed tissue.

36. The genetically modified immune cell of claim 35, wherein the chemokine receptor comprises CCR5, CXCR3, CCR4, CCR3, CCR6, CXCR3, CXCR4, CXCR5, or a combination thereof.

37. The genetically modified immune cell of any one of claims 21-36, wherein the immune cell is further modified to express or overly express an adhesion receptor.

38. The genetically modified immune cell of claim 37, wherein the adhesion receptor is involved in trafficking of pathologic immune cells to inflamed regions.

39. The genetically modified immune cell of claim 38, wherein the adhesion receptor comprises VLA-4, $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_L\beta_2$, or a combination thereof.

40. The genetically modified immune cell of any one of claims 21-39, further comprising a genetic modification that results in blockade of PD-1 signaling.

41. The genetically modified immune cell of claim 40, wherein the modification is a PD-1 knockout, a PD-L1 knockout, or a combination thereof.

42. The genetically modified immune cell of any one of claims 21-41, which is a regulatory T cell, wherein the regulatory T cell is CD25+.

43. The genetically modified immune cell of claim 42, wherein the regulatory T cell is derived from CD25++CD45R+ T cells isolated from peripheral blood mononuclear cells.

44. The genetically modified immune cell of claim 42, wherein the regulatory T cell comprises a transgene coding for CD25.

45. The genetically modified immune cell of any one of claims 42-44, wherein the regulatory T cell comprises a transgene coding for FoxP3.

46. The genetically modified immune cell of any one of claims 42-45, wherein the regulatory T cell further expresses a chimeric receptor specific to CD19, a chimeric receptor specific to CS-1, or both.

47. The genetically modified immune cell of any one of claims 42-46, wherein the regulatory T cell further express CCR6, CXCR5, PD-1, or a combination thereof.

48. The genetically modified immune cell of any one of claims 42-47, wherein the regulatory T cell is CD4+.

49. The genetically modified immune cell of any one of claims 42-48, wherein the regulatory T cell displays an antibody specific to an autoantigen.

50. The genetically modified immune cell of claim 49, wherein the autoantigen is an autoantigen listed in Table 1.

51. The genetically modified immune cell of claim 50, wherein the autoantigen is MOG.

52. The genetically modified immune cell of any one of claims 42-51, wherein the cell secretes an anti-inflammatory cytokine.

53. The genetically modified immune cell of 52, wherein the anti-inflammatory cytokine comprises IL-35, IL-10, and/or TGF-beta.

54. The genetically modified immune cell of any one of claims 21-41, which is a cytotoxic lymphocyte, wherein the cytotoxic T cell is CD8+.

55. The genetically modified immune cell of claim 54, wherein the cytotoxic T cell is derived from CD8+ T cells isolated from peripheral blood mononuclear cells.

56. The genetically modified immune cell of claim 54 or claim 55, wherein the cytotoxic T cell further expresses a chimeric receptor specific to CD19, a chimeric receptor specific to CS-1, or both.

57. The genetically modified immune cell of any one of claims 54-56, wherein the cytotoxic T cell further express CCR6, CXCR5, PD-1, or a combination thereof.

58. A method for suppressing autoreactive immune cells in a subject having an autoimmune disease, comprising administering to the subject an effective amount of genetically modified immune cells set forth in any one of claims 21-57.

59. The method of claim 58, wherein the genetically modified immune cells are T cells.

60. The method of claim 58 or claim 59, wherein the autoimmune disease is multiple sclerosis.

61. The method of any one of claims 58-60, wherein the genetically modified immune cells are autologous.

62. The method of any one of claims 58-60, wherein the genetically modified immune cells are allogenic.

63. The method of any one of claims 58-62, wherein the genetically modified immune cells are administered to a lymph node of the subject.

64. The method of any one of claims 58-63, wherein the subject is undergoing a therapy comprising an antibody specific to CD52.

65. The method of any one of claims 58-64, wherein the subject is a human patient having or at risk for multiple sclerosis and the genetically modified T cells are Treg cells as set forth in any one of claims 33-43.

66. The method of claim 65, wherein the human patient is an early-stage MS patient and the Treg cells express the MHC-CAR and have one or more of the following genetic modifications:

- (i) PD-L1 and/or PD-1 knockout;
- (ii) surface expression of CCR6 and/or CXCR5;
- (iii) surface display of an antibody or an antigen-binding fragment thereof that is

specific to MOG; and

- (iv) surface expression of a chimeric receptor targeting CD19.

67. The method of claim 66, wherein the human patient has relapsing-remitting MS or early stage progressive and the Treg cells express the MHC-CAR and have one or more of the following modifications:

- (i) surface expression of a chimeric receptor targeting CD19; and
- (ii) surface expression of CXCR5.

68. The method of claim 66, wherein the human patient has relapsing-remitting MS or early stage progressive and the Treg cells express the MHC-CAR and have one or more of the following modifications:

- (i) surface display of an antibody or an antigen-binding fragment thereof that is specific to MOG; and
- (ii) surface expression of CCR6.

69. The method of claim 66, where the human patient has MS in chronic progressive form and the Treg cells express the MHC-CAR and have one or more of the following modifications:

- (i) surface expression of a chimeric receptor targeting CS-1; and
- (ii) surface expression of an agent targeting CXCR4 and/or CXCR3.

70. The method of claim 66, wherein the human patient is an early-stage MS patient and the cytotoxic CD8 T cells express the MHC-CAR and have one or more of the following genetic modifications:

- (i) PD-L1 and/or PD-1 knockout;
- (ii) surface expression of CCR6 and/or CXCR5;
- (iii) surface display of an antibody or an antigen-binding fragment thereof that is specific to MOG; and
- (iv) surface expression of a chimeric receptor targeting CD19.

71. The method of any one of claims 58-67, wherein the subject is a human patient having or at risk for multiple sclerosis and the genetically modified T cells are

cytotoxic CD8+ T cells set forth in any one of claims 54-57.

72. The method of claim 71, wherein the human patient has relapsing-remitting MS or early stage progressive and the cytotoxic CD8 T cells express the MHC-CAR and have one or more of the following modifications:

- (i) surface expression of a chimeric receptor targeting CD19; and
- (ii) surface expression of CXCR5.

73. The method of claim 71, wherein the human patient has relapsing-remitting MS or early stage progressive and the cytotoxic CD8 T cells express the MHC-CAR and have one or more of the following modifications:

- (i) surface display of an antibody or an antigen-binding fragment thereof that is specific to MOG; and
- (ii) surface expression of CCR6.

74. The method of claim 71, where the human patient has MS in chronic progressive form and the cytotoxic CD8 T cells express the MHC-CAR and have one or more of the following modifications:

- (i) surface expression of a chimeric receptor targeting CS-1; and
- (ii) surface expression of an agent targeting CXCR4 and/or CXCR3.

75. The method of any one of claims 58-74, wherein the human patient is administered both the Treg cells set forth in any one of claims 33-43, and the cytotoxic cells set forth in any one of claims 54-57.

76. The method of claim 75, wherein the Treg cells and the cytotoxic cells are administered simultaneously or sequentially.

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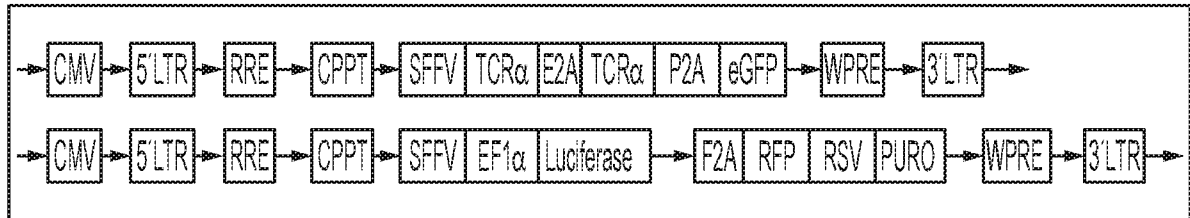


FIG. 1

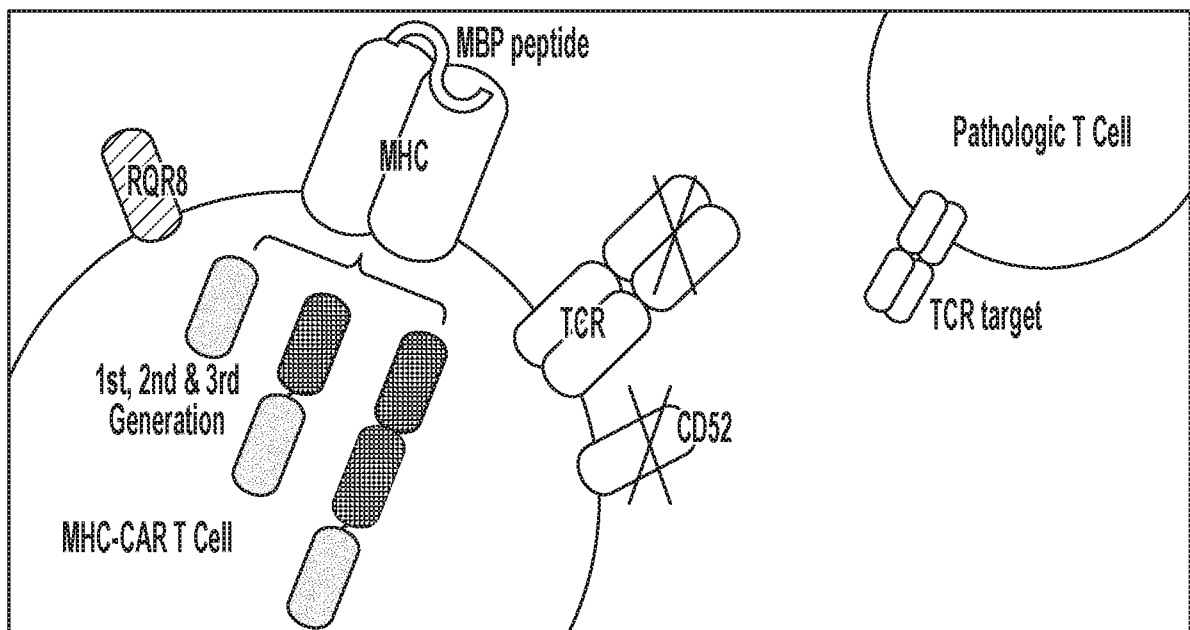


FIG. 2

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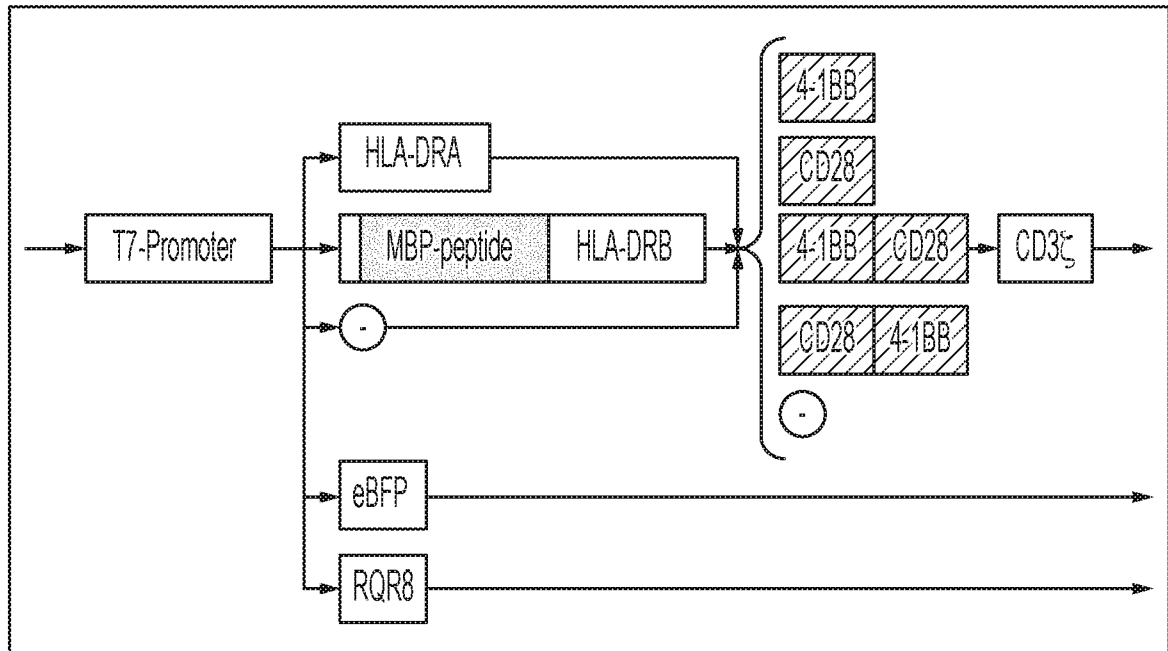


FIG. 3

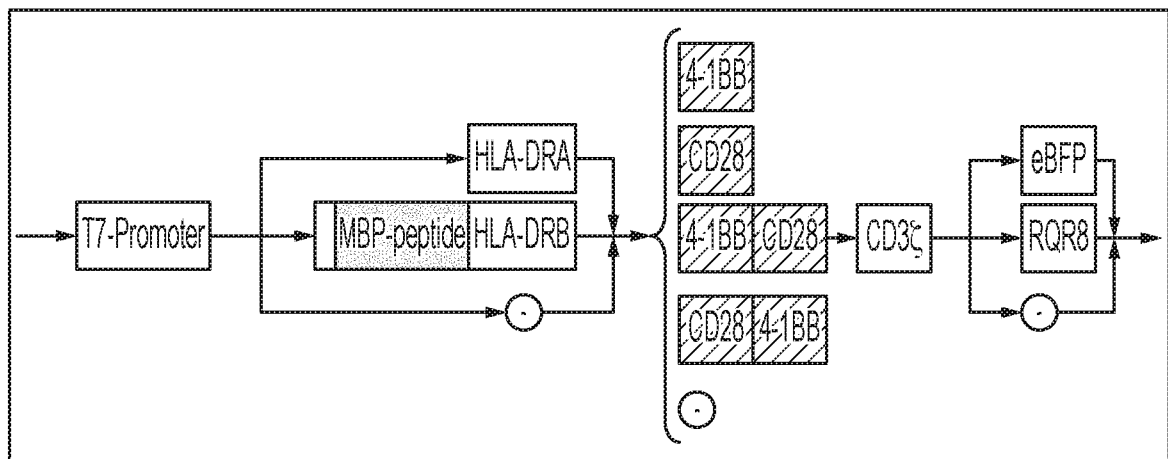


FIG. 4

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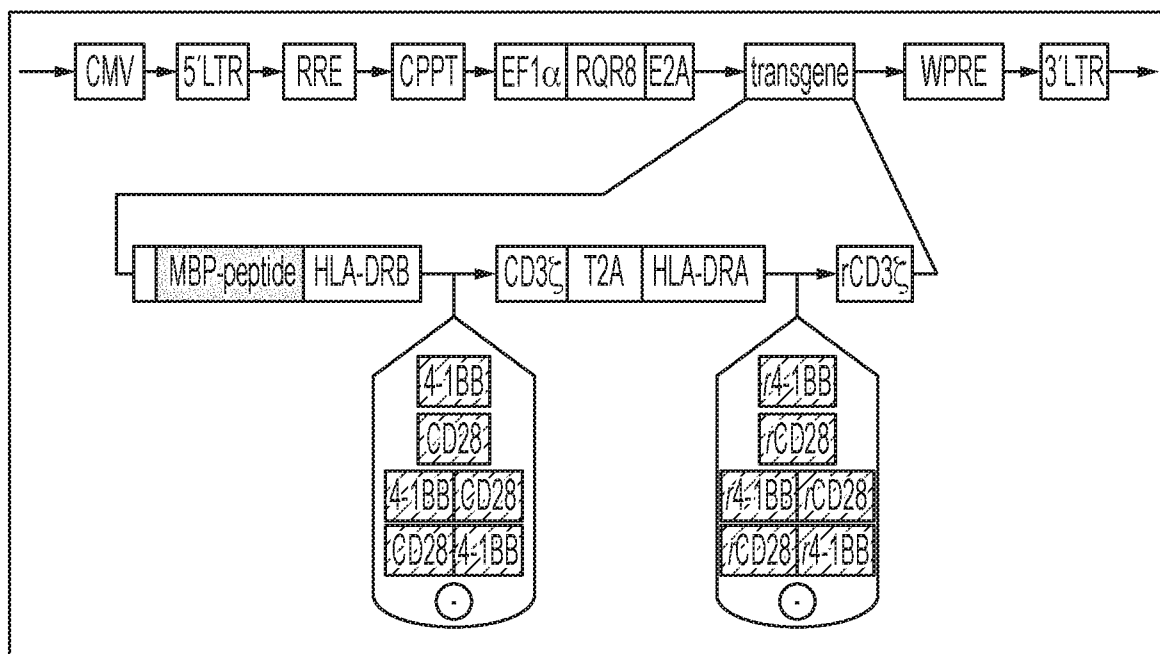


FIG. 5

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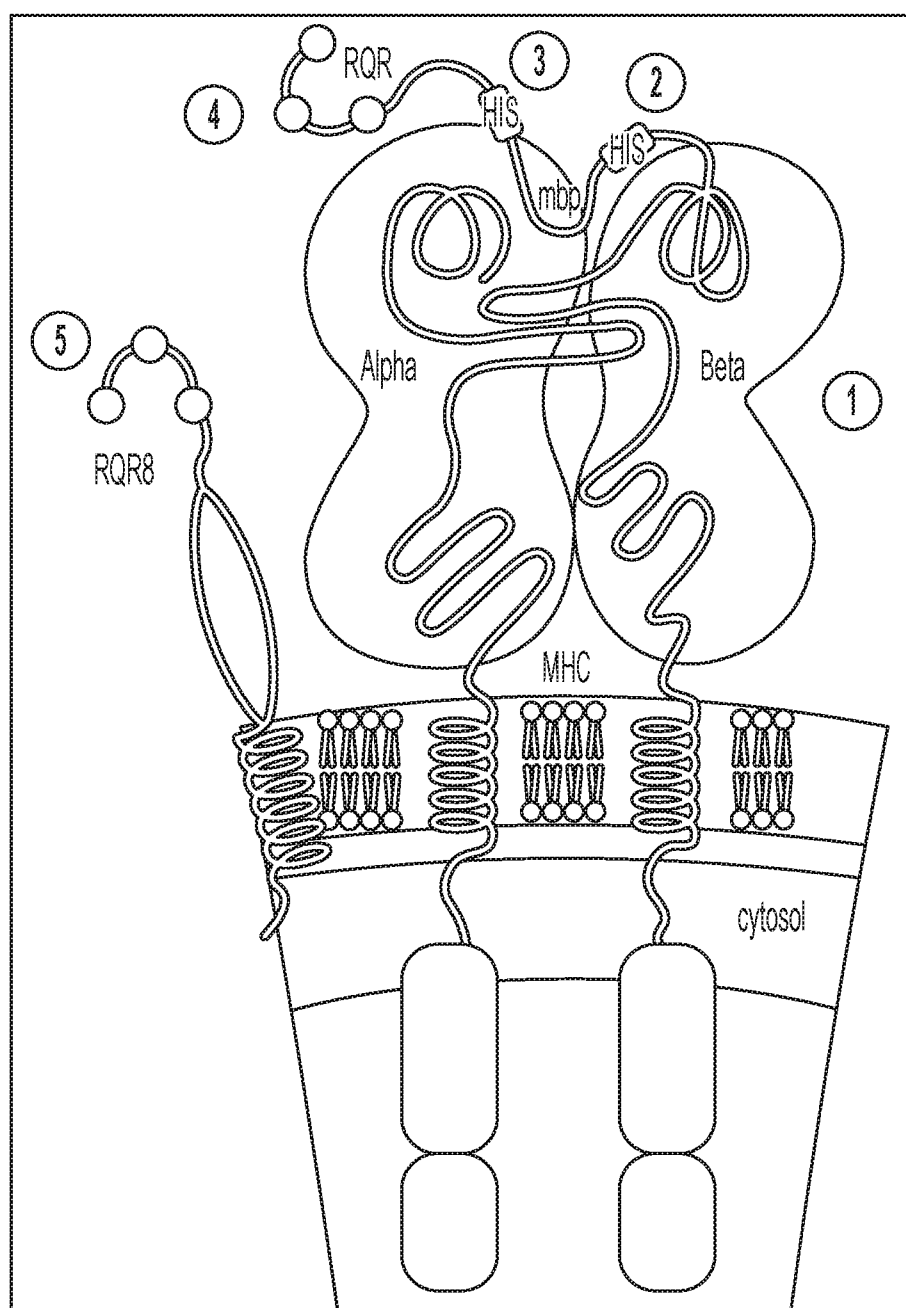


FIG. 6

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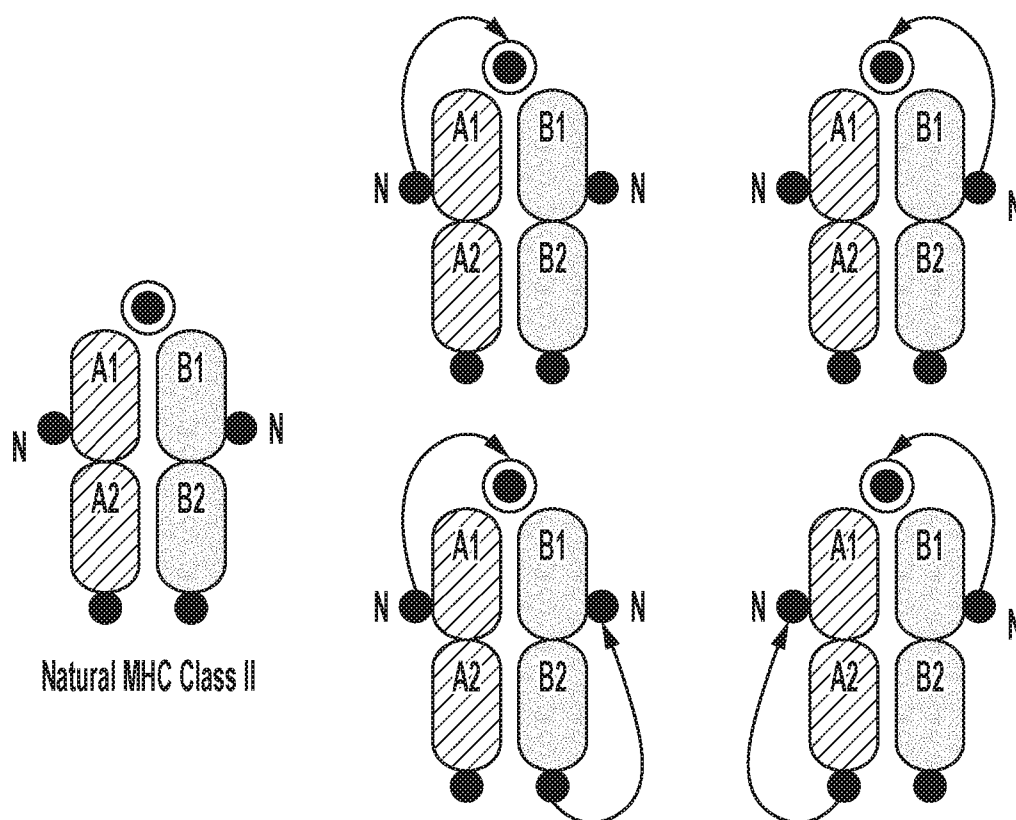


FIG. 7

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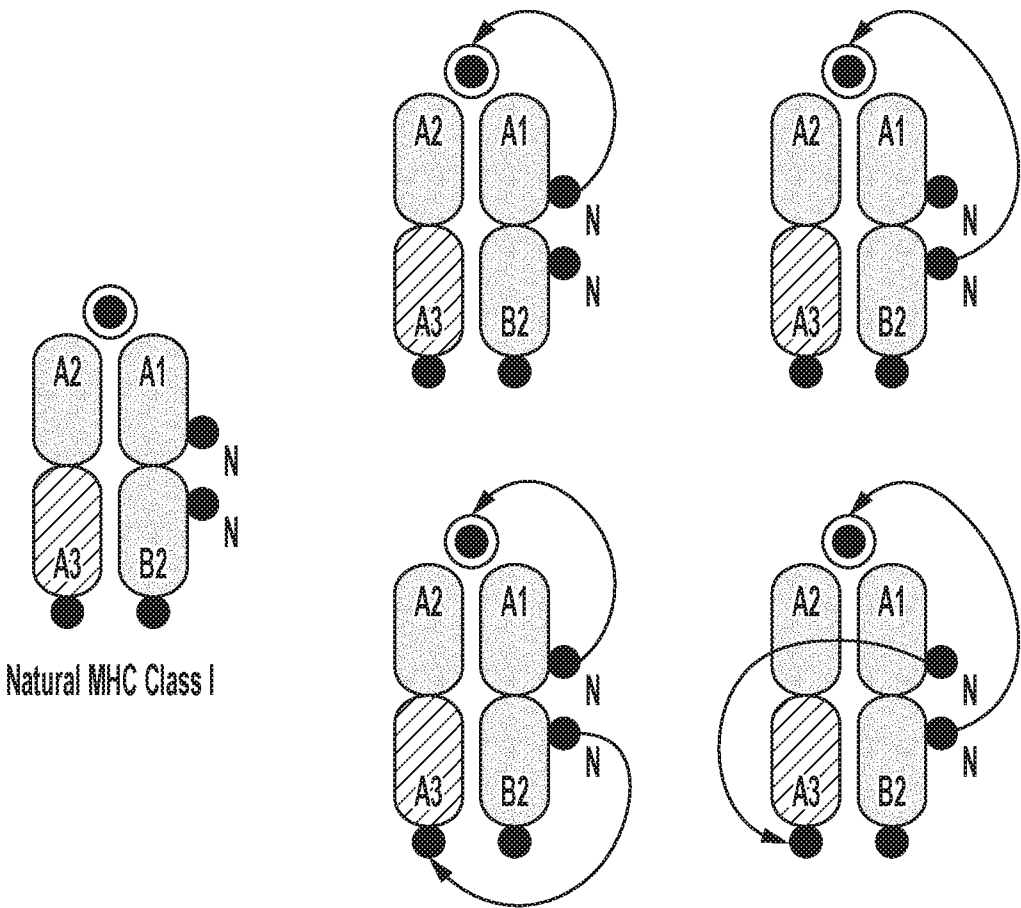


FIG. 8A

MHC Class I Exemplar Construct

Beta 2 -microglobulin signal	Linker 2	8-12 mer peptide	Linker 2 (G4S)3 or G6(SGG)3	Beta 2 -microglobulin Extracellular	Linker 3 (G4S)4 or (G4S)3	HLA-A	TM	41-BB or CD28	CD3 zeta
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FIG. 8B

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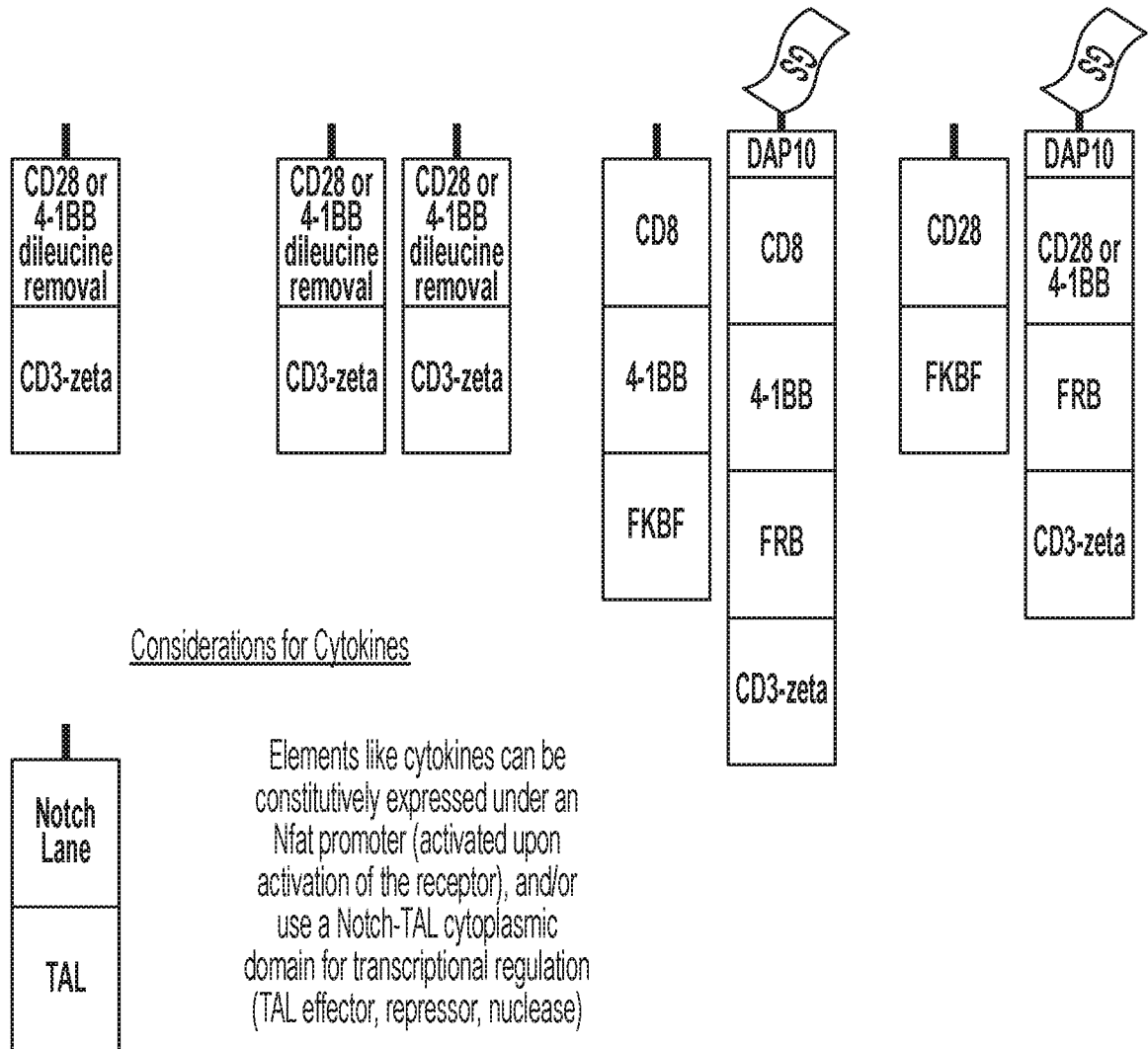
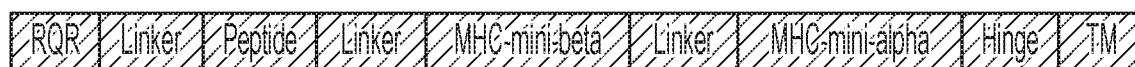


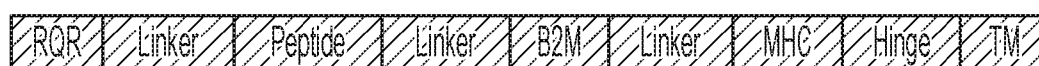
FIG. 9

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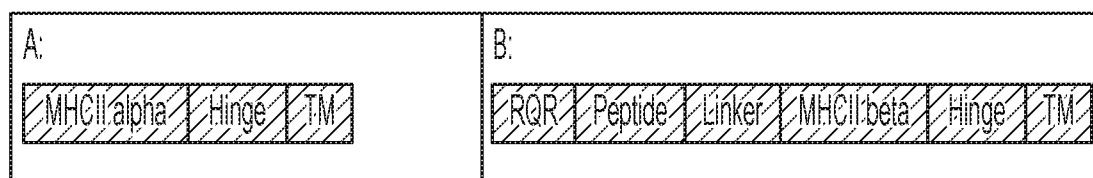
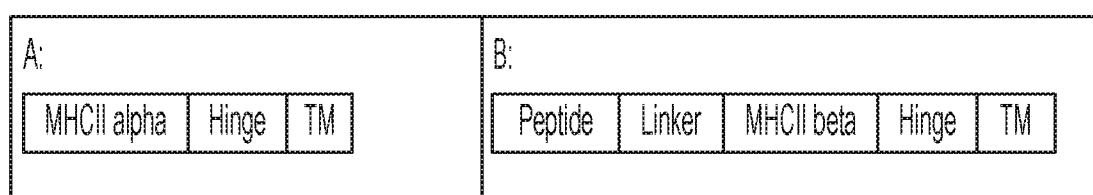
Single chain - Class II



Single chain - Class I



Multi-chain: Class II



Multi-chain: Class I

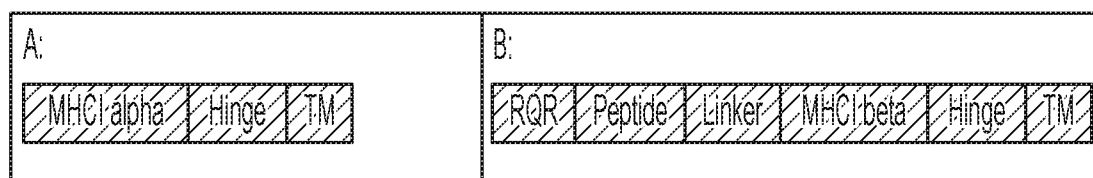
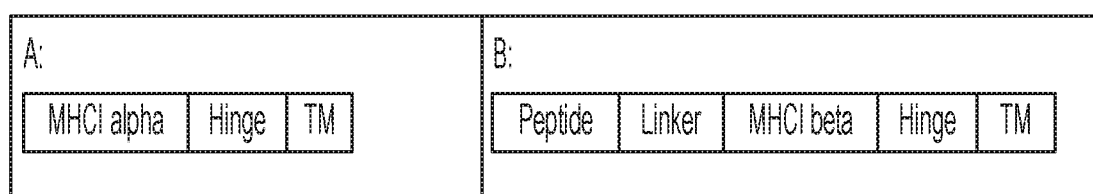


FIG. 10

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Multi-chain: Class I/II

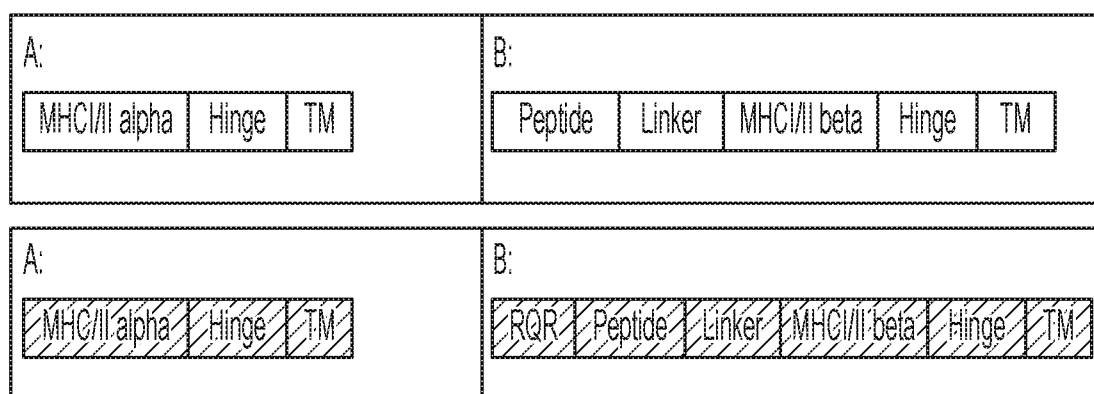
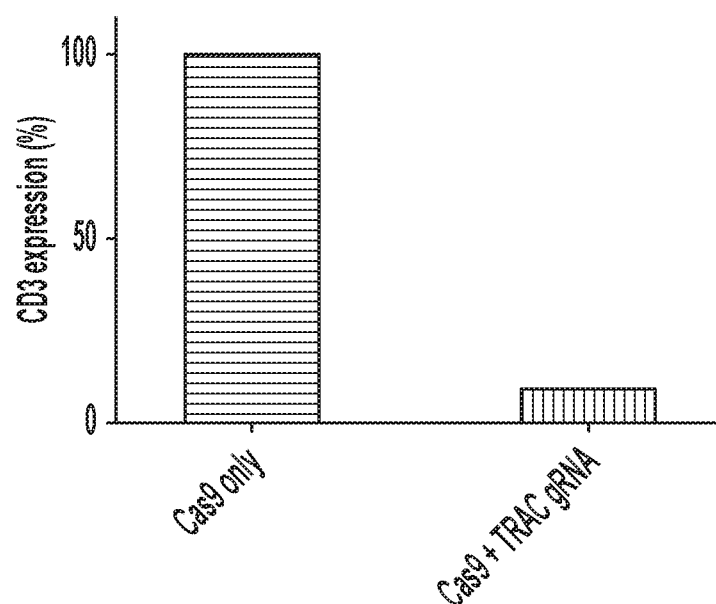
FIG. 10
continued

FIG. 11

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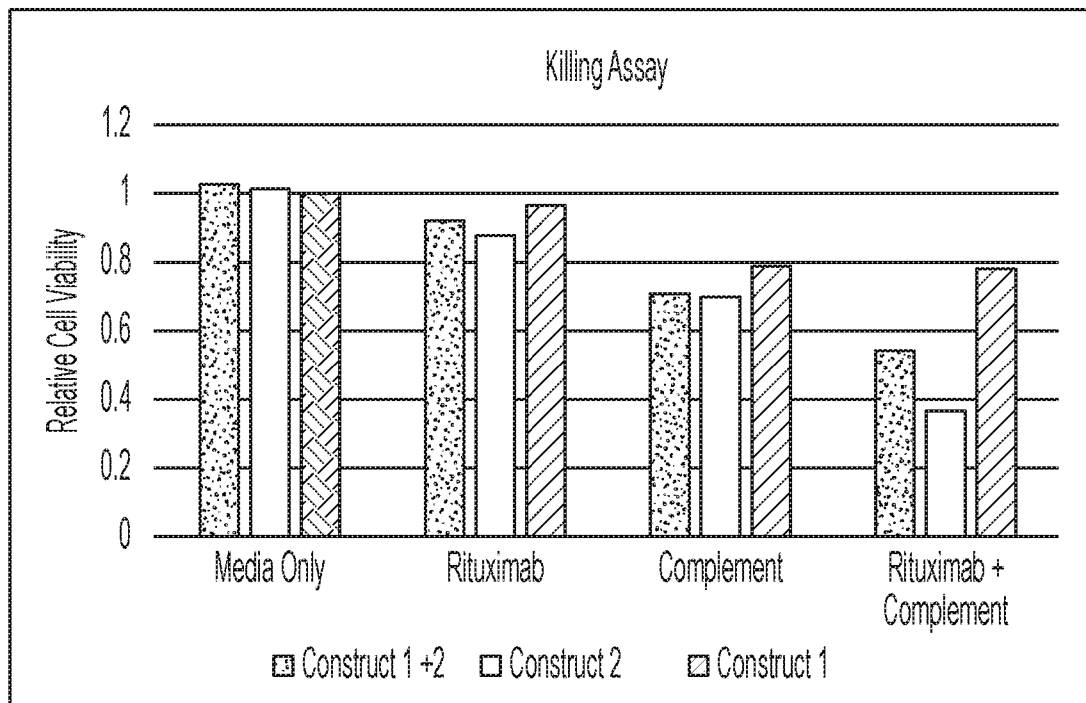


FIG. 12

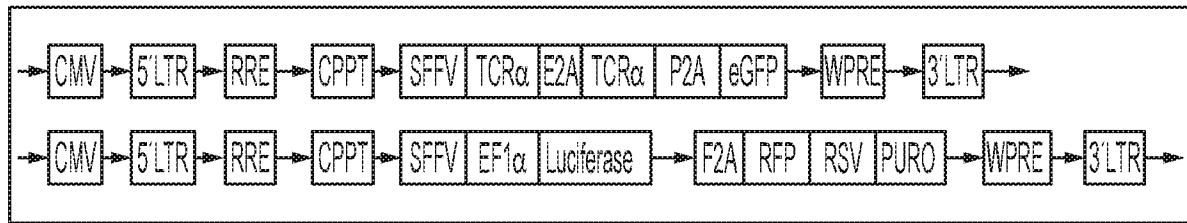


FIG. 1