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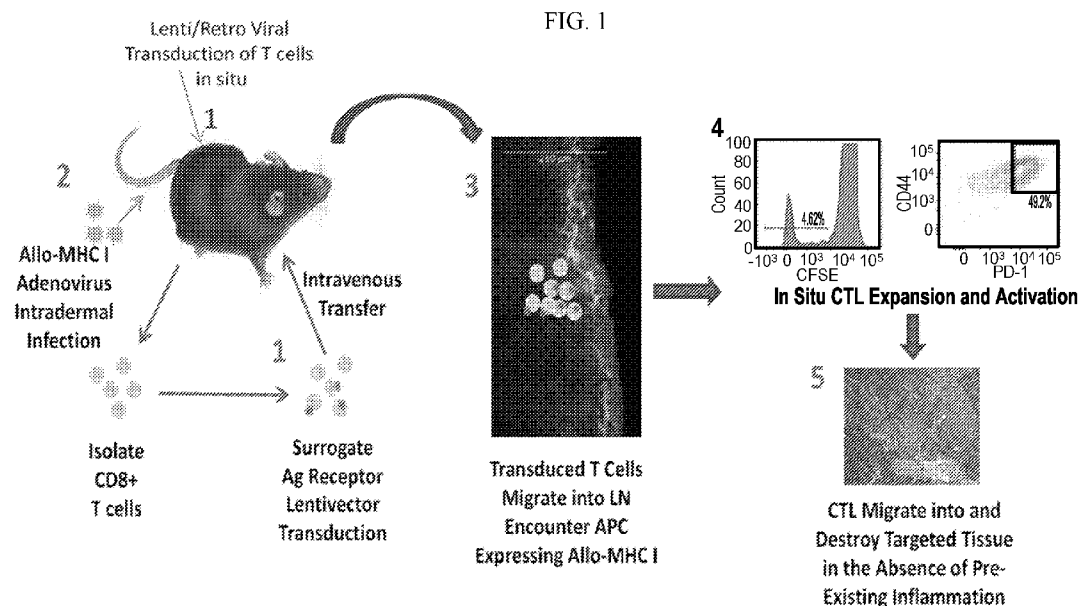
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(54) Title: MATERIALS AND METHODS FOR INCREASING IMMUNE RESPONSES



(57) Abstract: This document relates to materials and methods for activating nave T cells *in vivo*. For example, methods of activating nave T cells *in vivo* to treat cancer are provided.

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MATERIALS AND METHODS FOR INCREASING IMMUNE RESPONSES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial Nos. 62/618,399, filed on January 17, 2018, and 62/521,011, filed on June 16, 2017. The disclosures of the prior applications are considered part of the disclosure of this application, and are incorporated in their entirety into this application.

BACKGROUND

1. Technical Field

This document relates to materials and methods for activating naïve T cells *in vivo*. For example, *in vivo* activation of naïve T cells can be used to target cells (e.g., cancer cells) expressing a tumor antigen (e.g., a tumor-specific antigen).

2. Background Information

Approximately 22,000 people die from cancer each day globally. Cancers infiltrated by CD8⁺ T cells tend to have better prognoses than those devoid of these immune cells. However, effective antitumor cellular immunity is limited by the available T-cell receptor (TcR) repertoire consisting primarily of low affinity receptors specific for tumor associated antigens.

SUMMARY

This document provides materials and methods for activating naïve T cells (e.g., naïve T cells expressing tumor antigen receptors) *in vivo*. For example, naïve T cells expressing tumor-specific antigen receptors can be activated (e.g., to become cytotoxic T lymphocytes (CTLs)) *in vivo* by encountering antigens (e.g., antigens presented on an antigen presenting cell (APC) such as a subcapsular sinus macrophage and/or a dendritic cell) in a lymph node. The *in vivo* activated T cells can target cells (e.g., cancer cells) presenting the antigen (e.g., a tumor antigen) recognized by the tumor-specific antigen receptors. In some cases, the *in vivo* activated T cells can be expanded *in vivo*. Also provided herein are methods for using *in vivo* activation of naïve T cells as described herein (e.g., by *in vivo* activation of naïve T cells expressing tumor-specific antigen receptors). For example, *in vivo* activation of naïve T cells as described herein can be used to treat mammals (e.g., humans) having cancer.

As demonstrated herein, adoptively transferred naïve CD8⁺ T cells can migrate to a lymph node where they can encounter a virus (e.g., an adenovirus) encoding an allogeneic major histocompatibility complex class I (MHC I) antigen that can activate the naïve CD8⁺ T cells *in vivo*. Having the ability to activate naïve T cells expressing antigen receptors (e.g., tumor-specific antigen receptors) *in vivo* provides a unique and unrealized opportunity to generate CTLs capable of targeting (e.g., locating and destroying) cells (e.g., cancer cells) expressing a tumor antigen (e.g., a tumor-specific antigen) that can be recognized by the antigen receptor. For example, the ability to activate naïve T cells expressing tumor-specific antigen receptors *in vivo* provides the opportunity to target cancer cells, including cancer cells in solid tumors, that are otherwise undetectable by the immune system (e.g., cancers including quiescent cancer cells and/or cancers having escaped chemotherapy). In addition, the materials and methods described herein can be more conducive to “off the shelf” reagents. As such, personalized therapies in the form of tumor-specific immune responses can be rapidly and efficiently applied to wide patient populations while limiting costs.

As also described herein, using a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide) to activate naïve T cells within a mammal can result in the activation of many different naïve T cells within the mammal, thereby producing a polyclonal T cell response in the mammal. In some case, a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide) can be used to activate more than 1, 2.5, 5, 10, 15, or 20 percent of the naïve T cells within a mammal or can be used to activate more than 1, 2.5, 5, 10, 15, or 20 percent of the naïve T cells within a lymph node of a mammal. In addition, the CD8⁺ T cells that are activated *in vivo* using a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide) can be potent killers of target cells recognized by those activated CD8⁺ T cells. This level of target cell killing can be greater than that observed by comparable CD8⁺ T cells that are activated *in vitro*.

As further described herein, the naïve T cells that are activated using a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide) as described herein can be engineered (e.g., engineered *in vivo* or *in vitro*) to express an antigen receptor to a desired target before (or, for *in vivo* approaches, after or at the same time as) being activated. For example, when engineering naïve T cells *in*

vivo, a vector (e.g., a viral vector such as a lentiviral vector or retroviral vector) encoding an antigen receptor (e.g., a chimeric antigen receptor such as a chimeric antigen receptor specific for a tumor antigen) can be administered to the mammal (e.g., a human) before the mammal is administered a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide), after the mammal is administered a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide), or at the same time that the mammal is administered a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide). In some cases, when engineering naïve T cells *in vivo*, a vector (e.g., a viral vector such as a lentiviral vector or retroviral vector) encoding an antigen receptor (e.g., a chimeric antigen receptor such as a chimeric antigen receptor specific for a tumor antigen) can be administered to the mammal (e.g., a human) before and after the mammal is administered a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide). In some cases, when engineering naïve T cells *in vivo*, a vector (e.g., a viral vector such as a lentiviral vector or retroviral vector) encoding an antigen receptor (e.g., a chimeric antigen receptor such as a chimeric antigen receptor specific for a tumor antigen) can be administered to the mammal (e.g., a human) before, after, and at the same time the mammal is administered a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide).

In cases when naïve T cells are engineered *in vitro*, a vector (e.g., a viral vector such as a lentiviral vector or retroviral vector) encoding an antigen receptor (e.g., a chimeric antigen receptor such as a chimeric antigen receptor specific for a tumor antigen) can be introduced into *in vitro* naïve T cells obtained from a mammal (e.g., a human) and introduced back into that mammal before that mammal is administered a virus (e.g., an adenovirus) designed to express an MHC I polypeptide (e.g., an allogeneic MHC I polypeptide). In some cases, the *in vitro* naïve T cells can be treated with one or more agents designed to stimulate the cells (e.g., anti-CD3 agents, anti-CD38 agents, interleukin (IL) 2, IL15, or combinations thereof) before, after, or both before and after the vector is introduced into the cells.

When applying the methods and materials described herein specifically to humans or human cells, the MHC I polypeptides described herein can be referred to as HLA

polypeptides (e.g., HLA-A, HLA-B, and/or HLA-C polypeptides) or human MHC I polypeptides.

In general, one aspect of this document features a method for activating a naïve T cell in a mammal. The method includes, or consists essentially of, engineering a naïve T cell to express an antigen receptor, thereby forming an engineered naïve T cell, and activating the engineered naïve T cell in the mammal. The mammal can be a human. The naïve T cell can be a naïve cytotoxic T lymphocyte. The antigen receptor can be a chimeric antigen receptor. The antigen receptor can be a tumor-specific or antigen receptor. In some cases, the engineering can include *ex vivo* engineering. The *ex vivo* engineering can include obtaining the naïve T cell from the mammal, introducing nucleic acid encoding the antigen receptor into the naïve T cells to produce the engineered naïve T cell, and administering the engineered naïve T cells to the mammal. The introducing can include transducing the naïve T cells with a viral vector encoding the antigen receptor. The viral vector can be a lentiviral vector or a retroviral vector. The administering can include intravenous injection. In some cases, the engineering can include *in situ* engineering. The *in situ* engineering can include administering a viral vector encoding the antigen receptor to the mammal. The administering can include intradermal injection. The intradermal injection can be directly into a lymph node. The viral vector can be an adenoviral vector. The activating the engineered naïve T cell *in vivo* can include administering a viral vector encoding an antigen to the mammal. The antigen can be an alloantigen. The alloantigen can be an allogeneic major histocompatibility complex class I antigen. The viral vector can be an adenoviral vector. The administering can include intradermal injection. The intradermal injection can be directly into a lymph node.

In another aspect, this document features a method for treating a mammal having cancer. The method includes, or consists essentially of, engineering a naïve T cell to express a tumor-specific antigen receptor, thereby forming an engineered naïve T cell, and activating the engineered naïve T cell *in vivo*. The mammal can be a human. The cancer can be acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), chronic myelogenous leukemia (CML), acute monocytic leukemia (AMOL)), Hodgkin's lymphoma, non-Hodgkin's lymphoma, myelomas, ovarian cancer, breast cancer, prostate cancer, or colon cancer. The cancer can include cancer cells expressing a tumor-specific

antigen. The naïve T cell can be engineered to express a tumor-specific antigen receptor that targets the tumor-specific antigen. The tumor-specific antigen can be mucin 1 (MUC-1), human epidermal growth factor receptor 2 (HER-2), or estrogen receptor (ER). In some cases, the engineering can include *ex vivo* engineering. The *ex vivo* engineering can include obtaining the naïve T cells from the mammal, introducing nucleic acid encoding the antigen receptor into the naïve T cells to produce the engineered naïve T cell, and administering the engineered naïve T cells to the mammal. The introducing can include transducing the naïve T cells with a viral vector encoding the antigen receptor. The viral vector can be a lentiviral vector. The administering can include intravenous injection. The administering can include administering from about 200 to about 1500 engineered naïve T cells (e.g., about 300 engineered naïve T cells) to the mammal. In some cases, the engineering can include *in situ* engineering. The *in situ* engineering can include administering a viral vector encoding the antigen receptor to the mammal. The administering can include intradermal injection. The intradermal injection can be directly into a lymph node. The viral vector can be an adenoviral vector. The activating the engineered naïve T cell *in vivo* can include administering a viral vector encoding an antigen to the mammal. The antigen can be an alloantigen. The alloantigen can be an allogeneic major histocompatibility complex class I antigen. The viral vector can be an adenoviral vector. The administering can include intradermal injection. The intradermal injection can be directly into a lymph node. The cancer can include solid tumors. The cancer can be in remission. The cancer can include quiescent cancer cells. The cancer can include cancer cells that escaped chemotherapy or are non-responsive to chemotherapy.

In another aspect, this document features a method for obtaining an activated T cell within a mammal where the activated T cell includes a heterologous antigen receptor. The method includes, or consists essentially of, (a) introducing nucleic acid encoding a heterologous antigen receptor into T cells obtained from a mammal *in vitro* to obtain engineered T cells, (b) administering the engineered T cells to the mammal, and (c) administering a virus including nucleic acid encoding an MHC class I polypeptide to the mammal; where an engineered T cell of the engineered T cells administered to the mammal in step (b) is activated. The mammal can be a human. The T cells obtained from the mammal can be naïve T cells. The naïve T cells can be naïve cytotoxic T lymphocytes. The antigen receptor can be a chimeric antigen receptor. The antigen

receptor can be a tumor-specific antigen receptor. The nucleic acid encoding the heterologous antigen receptor can be introduced into the T cells with a viral vector comprising the nucleic acid. The viral vector can be a lentiviral vector. The engineered T cells can be administered to the mammal via intravenous injection. The engineered T
5 cells can be administered to the mammal via injection into a lymph node of the mammal. The virus can be an adenovirus or a rhabdovirus. The virus can be administered to the mammal via intradermal injection. The virus can be administered to the mammal via direct administration into a lymph node of the mammal. The MHC class I polypeptide can be an allogeneic MHC class I polypeptide. The MHC class I polypeptide can be an
10 HLA-A, HLA-B, or HLA-C polypeptide. The engineered T cell activated within the mammal in step (c) can include a native T cell receptor. Step (c) can activate a plurality of engineered T cells within the mammal. The activated T cells of the plurality of engineered T cells can include different native T cell receptors.

In another aspect, this document features a method for obtaining an activated T
15 cell within a mammal where the activated T cell includes a heterologous antigen receptor. The method includes, or consists essentially of, administering to a mammal (a) nucleic acid encoding a heterologous antigen receptor and (b) a virus comprising nucleic acid encoding an MHC class I polypeptide, where the nucleic acid is introduced into T cells within the mammal to form engineered T cells including the heterologous antigen
20 receptor, where administration of the virus activated T cells within the mammal, and where at least one T cell within the mammal includes the heterologous antigen receptor and is activated. The mammal can be a human. The at least one T can be a cytotoxic T lymphocyte. The antigen receptor can be a chimeric antigen receptor. The antigen receptor can be a tumor-specific antigen receptor. The nucleic acid encoding the
25 heterologous antigen receptor can be introduced into the T cells with a viral vector including the nucleic acid. The viral vector can be a lentiviral vector or retroviral vector. The nucleic acid can be administered to the mammal via intravenous injection. The nucleic acid can be administered to the mammal via injection into a lymph node of said mammal. The virus can be an adenovirus or a rhabdovirus. The virus can be
30 administered to the mammal via intradermal injection. The virus can be administered to the mammal via direct administration into a lymph node of the mammal. The nucleic acid can be administered to the mammal before the virus is administered to the mammal. The nucleic acid encoding the heterologous antigen receptor can be introduced into the T

cells with a lentiviral vector including the nucleic acid. The nucleic acid can be administered to the mammal after the virus is administered to the mammal. The nucleic acid encoding the heterologous antigen receptor can be introduced into the T cells with a retroviral vector including the nucleic acid. The MHC class I polypeptide can be an allogeneic MHC class I polypeptide. The MHC class I polypeptide can be an HLA-A, HLA-B, or HLA-C polypeptide. The at least one T cell can include a native T cell receptor. The at least one T cell can be a plurality of activated T cells including the heterologous antigen receptor. The activated T cells of the plurality of the activated T cells can include different native T cell receptors.

In another aspect, this document features an isolated virus including nucleic acid encoding an MHC class I polypeptide. The virus can be a picornavirus, an adenovirus, or a rhabdovirus (e.g., a vesicular stomatitis virus). The virus can be replication-defective. The MHC class I polypeptide can be a human MHC class I polypeptide. The MHC class I polypeptide can include the amino acid sequence set forth in SEQ ID NO:4.

In another aspect, this document features a kit having a first container including a first virus including nucleic acid encoding an antigen receptor and a second container including a second virus including nucleic acid encoding an MHC class I polypeptide. The first virus can be a lentivirus or a retrovirus. The antigen receptor can be a chimeric antigen receptor. The second virus can be a picornavirus, an adenovirus, or a rhabdovirus (e.g., a vesicular stomatitis virus). The second virus can be replication-defective. The MHC class I polypeptide can be a human MHC class I polypeptide. The MHC class I polypeptide can include the amino acid sequence set forth in SEQ ID NO:4.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and

advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows an exemplary scheme for *in vivo* activation of naïve T cells expressing surrogate antigen receptors. (1) Isolated CD8+ T cells are transduced with lentivirus or retrovirus encoding surrogate receptors are adoptively transferred intravenously back into a host (bottom), or T cells are transduced *in situ* in draining lymph nodes (top). (2) Allogeneic MHC I (allo-MHC I) is expressed by adenovirus introduced intradermally. (3) Transduced T cells migrate into lymph nodes and encounter APC expressing allo-MHC I. (4) Allo-reactive CTLs are activated, and (5) leave lymph node and destroy cells expressing antigens targeted by surrogate receptors.

Figures 2A and 2B shows that normal tissue was targeted and destroyed by virus activated tissue-specific CTL. 1200 OT-1 T cells were adoptively transferred into RIP-OVA mice, then activated with TMEV-OVA. Figure 2A contains photographs of haemotoxylin and Eosin (H&E) staining and immunohistochemistry (IHC) staining for insulin showing pancreatic inflammation within 5 days of CTL induction by virus. Figure 2B contains a graph showing significant destruction of islets at day 21 in surviving mice. No virus was detected in pancreas by PCR. The pancreas was totally destroyed with increased numbers of OT-1 cells. Similar results were observed when replication defective adenovirus encoding ovalbumin was used to induce pancreas destruction by OT-1 T cells.

Figures 3A – 3C are photographs of fluorescent microscopy showing transduction of lymph node (LN) cells. mTmG-mice were infected by intradermal infection with an adenovirus expressing a cre recombinase (adeno-cre). Figure 3A shows that adeno control virus infected LN cells. Figure 3B shows that the adeno-cre infected LN and expressed cre recombinase in the LN. Figure 3C shows a low magnification view of LN showing marginal location of transduced cells.

Figure 4A is a schematic of an exemplary replication-defective adenovirus (serotype 6) vector expressing a mutant MHC molecule, which functions as a universal alloantigen. Figure 4B is a generic version of the vector construct, by using an engineered mutant MHC molecule, the MHC can be universally allogeneic to any person. Alternatively, by using a naturally occurring MHC class I molecule, the MHC can be allogeneic to a cohort or subset of a population.

Figure 5 contains dot plots showing that allo-reactive CTLs were generated in response to adenovirus encoding allogeneic MHC I. Allo-MHC I adenovirus was introduced into LN by intradermal injection. Four days later, syngeneic (BALB/c), allogeneic (B6), and third party (C3H) labeled target cells were adoptively transferred intravenously into challenged hosts in an *in vivo* CTL assay. Four hours later spleen cells were harvested and analyzed by flow cytometry for the presence of introduced target cells. B6 target cells (targets expressing introduced allo-MHC I) were completely eliminated *in vivo*.

Figure 6 contains dot plots showing that adoptively transferred CD8⁺ T cells responded to adeno-alloMHC I. Freshly isolated syngenic CD8⁺ T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) before transfer, followed by challenge with adeno-allo-MHC I or control virus. Figures 6A and 6C show that adoptively transferred CFSE-labeled T cells migrate to the LN where they encounter and respond to transduced allo-MHC I molecules. Figure 6C also shows that the stimulated cells proliferate when stimulated with allo-MHC I, diluting the CFSE. Figures 6B and 6D shows that the CFSE-dilute population displayed a more activated phenotype expressing high CD44 and PD-1 (D) relative to the CFSE-dilute cells isolated from lymph nodes challenged with control adenovirus (B).

Figure 7 is a photograph of fluorescent microscopy showing lentivirus transduction of naïve CD8⁺ spleen cells from a mTmG-reporter mouse. CD8⁺ enriched naïve spleen cells were transduced with lentivirus-cre. The cells were subsequently activated with anti-CD3/CD28+ IL-2 to maintain viability in culture for 4 days. Successful transduction results in the transition from red to green fluorescence.

Figure 8 is a dot plot showing successful *in situ* introduction of transgene into activated lymph node cells. Adenoviral vector encoding alloMHC was injected intradermally into mTmG reporter mice to stimulate draining lymph node, four days later lentivirus-cre was directly injected into the enlarged lymph node. After 24 hours, CD8⁺ T cells from the lymph node were harvested and cultured for 3 days in the presence of IL2+IL7 to allow membrane eGFP expression.

Figure 9 contains dot plots showing successful transduction of transgene into human cells. Human CAR lentiviral vector effective at transducing human, but not mouse T cells.

Figure 10 contains photographs showing intradermal introduction of non-replicating virus. Hu-NSG mice lack lymph nodes. Evans blue injected intradermally in the tail to mark inguinal lymph node in WT, NOD Scid IL-2R $\gamma^{-/-}$ (NSG), and human CD34⁺ hematopoietic cell reconstituted NSG mice (hu-NSG).

5 Figure 11 contains dot plots showing alternative routes of administration for in vivo CTL. All three immunization routes were effective as indicated by the relative depletion of the B6 target cells.

Figure 12 shows an exemplary scheme for using hu-NSG hosts. (1) Human B cells circulating in the hu-NSG host are assessed. (2) T cells from the spleen of the nu-NSG host are contacted with a lentivirus encoding a target antigen, and injected
10 intravenously into the nu-NSG host mouse. Replication defective adenovirus 6 encoding the MHC allogeneic antigen H-2K^b are injected intravenously and an identical dose was injected intraperitoneally. (3) 1 week after treatment, the composition of human B cells in the blood is assessed.

15 Figure 13 contains graphs showing human leukocyte composition prior to experiment of hu-NSG mice.

Figure 14 contains graphs showing *in vivo* CTL activates human immune cells in hu-NSG hosts. The expected 1:1 ratio of recovered target cells was altered in all three recipients indicating a preferential killing of the K^b⁺ spleen cells (panel A). The ratio of
20 recovered K^b⁺ cells was significantly lower relative to the K^b⁻ target cells (panel B).

Figure 15 contains a graph showing raw data of the drop in B cell numbers in hu-NSG mice receiving CART treatment and AD6 vaccination.

Figure 16 contains graphs showing normalized change in CD19⁺ B cells following introduction of Ad6-*alloMHC* (K^b) and lenti-CAR19 transduced spleen cells
25 from hu-NSG mice reconstituted with CD34⁺ cells from the identical human donor.

^aStatistical evaluation normalized to account for the depletion of peripheral blood cell populations in the mice caused by repetitive blood sampling. *Increase in T cells following therapy is consistent with previous CART therapy findings.

Figure 17 contains a sequence listing of a nucleic acid sequence (SEQ ID NO:1) encoding a human MHC I polypeptide (an HLA-B40:28) and the amino acid sequence
30 (SEQ ID NO:3) of that human MHC I polypeptide, and a sequence listing of a nucleic acid sequence (SEQ ID NO:2) encoding a human MHC I polypeptide (an HLA-

DRB1*12:01:01:01) and the amino acid sequence (SEQ ID NO:4) of that human MHC I polypeptide.

DETAILED DESCRIPTION

This document provides materials and methods for activating naïve T cells (e.g.,
 5 naïve T cells expressing tumor-specific antigen receptors) *in vivo* (e.g., making *in vivo* activated CTLs). For example, naïve T cells expressing tumor-specific antigen receptors can be activated (e.g., to become CTLs) *in vivo* by encountering antigens (e.g., antigens presented on an APC such as a subcapsular sinus macrophage and/or a dendritic cell) in a lymph node. *In vivo* activated CTLs can include effector T cells and/or memory T cells.
 10 In some cases, naïve T cells can be engineered to express tumor-specific antigen receptors *ex vivo*. For example, naïve T cells can be obtained, engineered *ex vivo* to express tumor-specific antigen receptors, and administered (e.g., by adoptive transfer) to a mammal. Adoptively transferred naïve T cells can migrate to one or more lymph nodes to be activated *in vivo*. In some cases, naïve T cells can be engineered to express tumor-
 15 specific antigen receptors *in situ*. For example, expression vectors (e.g., viral vectors) can be injected into secondary lymphoid organs such that naïve T cells are engineered *in situ* to express tumor-specific antigen receptors. When the naïve T cells expressing tumor-specific antigen receptors encounter an antigen (e.g., an antigen presented by an APC such as a subcapsular sinus macrophage and/or a dendritic cell), the naïve T cells are
 20 activated (e.g., to become CTLs) *in vivo*. The *in vivo* activated T cells can target cells (e.g., cancer cells) expressing the antigen (e.g., a tumor antigen) recognized by the tumor-specific antigen receptors. In some cases, the *in vivo* activated T cells can target cancer cells in tissues that lack current and/or preexisting inflammation. In some cases, the *in vivo* activated T cells do not target normal (e.g., healthy a non-cancerous) cells.
 25 A naïve T cell that can be activated *in vivo* as described herein can be any appropriate naïve T cell. Examples of naïve T cells include, without limitation, CTLs (e.g., CD4+ CTLs and/or CD8+ CTLs). For example, a naïve T cell that can be activated *in vivo* as described herein can be a CD8+ CTL. In some cases, one or more naïve T cells can be obtained from a mammal (e.g., a mammal having cancer). For example, naïve T
 30 cells can be obtained from a mammal to be treated with the materials and method described herein.

A naïve T cell activated *in vivo* as described herein can express (e.g., can be engineered to express) any appropriate antigen receptor. In some cases, an antigen

receptor can be a heterologous antigen receptor. In some cases, an antigen receptor can be a chimeric antigen receptor (CAR). In some cases, an antigen receptor can be a tumor antigen (e.g., tumor-specific antigen) receptor. For example, a naïve T cell can be engineered to express a tumor-specific antigen receptor that targets a tumor antigen (e.g., a cell surface tumor antigen) expressed by a cancer cell in a mammal having cancer. In some cases, an antigen receptor can be an indirect antigen receptor. For example, a naïve T cell can be engineered to express an indirect antigen receptor that targets a first antigen (e.g., an exogenous antigen). In some cases, a target cell (e.g., a cancer cell in a mammal having cancer) can express a first antigen (e.g., a tumor antigen) can be recognized by a reagent (e.g., an antibody) containing a second antigen, and a naïve T cell can be engineered to express an antigen receptor that targets the second antigen. In some cases, a tumor antigen can be a tumor-specific antigen (TSA; e.g., a tumor antigen present only on tumor cells). In some cases, a tumor antigen can be a tumor-associated antigen (TAA; e.g., an abnormal protein present on tumor cells). Examples of tumor antigens that can be recognized by a tumor antigen receptor expressed in a naïve T cell include, without limitation, mucin 1 (MUC-1), human epidermal growth factor receptor 2 (HER-2), estrogen receptor (ER), epidermal growth factor receptor (EGFR), folate receptor alpha, and mesothelin. As described herein, a naïve T cell can be engineered to have an antigen receptor (e.g., a heterologous antigen receptor) that recognizes any appropriate antigen. In some cases, a naïve T cell can be engineered to have an antigen receptor (e.g., a heterologous antigen receptor) that recognizes persistent virus antigens or senescent cells.

Any appropriate method can be used to express an antigen receptor on a naïve T cell. For example, a nucleic acid encoding an antigen receptor can be introduced into the one or more naïve T cells. In some cases, viral transduction can be used to introduce a nucleic acid encoding an antigen receptor into a non-dividing cell. A nucleic acid encoding an antigen receptor can be introduced in a naïve T cell using any appropriate method. In some cases, a nucleic acid encoding an antigen receptor can be introduced into a naïve T cell by transduction (e.g., viral transduction using a retroviral vector or a lentiviral vector) or transfection. In some cases, a nucleic acid encoding an antigen receptor can be introduced *ex vivo* into one or more naïve T cells. For example, *ex vivo* engineering of naïve T cells expressing an antigen receptor can include transducing isolated naïve T cells with a lentiviral vector encoding an antigen receptor. In cases where naïve T cells are engineered *ex vivo* to express an antigen receptor, the naïve T

cells can be obtained from any appropriate source (e.g., a mammal such as the mammal to be treated or a donor mammal, or a cell line). In some cases, a nucleic acid encoding an antigen receptor can be introduced into one or more naïve T cells *in situ* into the lymphatic system (e.g., into one or more secondary lymphoid organs such as the lymph nodes and the spleen). For example, *in situ* engineering of naïve T cells to express an antigen receptor can include intradermal (ID) injection (e.g., directly into one or more lymph nodes) of a lentiviral vector encoding an antigen receptor.

Any appropriate method can be used to activate the naïve T cells described herein (e.g., engineered naïve T cells such as naïve T cells designed to express tumor-specific antigen receptors). For example, naïve T cells expressing tumor-specific antigen receptors can be activated *in vivo* by administering one or more immunogens (e.g., antigens) to a mammal. Any appropriate immunogen can be used to activate a naïve T cell described herein. In some cases, an immunogen can be a cell surface antigen (e.g., a cell surface antigen expressed by a cancer cell). In some cases, an immunogen can be an allogeneic immunogen (e.g., an allogeneic antigen (also referred to as an alloantigen)). Examples of antigens that can be used to activate the naïve T cells described herein include, without limitation, an allogeneic MHC class I polypeptide (allo-MHC I or alloMHC I polypeptide) and an allogeneic MHC class II polypeptide (allo-MHC II or alloMHC II polypeptide). Such antigens can be presented as one or more fragments in the context of an MHC molecule such as MHC I. For example, naïve T cells expressing tumor-specific antigen receptors can be activated *in vivo* by administering allo-MHC I to a mammal.

Any appropriate method can be used to administer an immunogen (e.g., an antigen) to a mammal (e.g., a human). Examples of methods of administering immunogens to a mammal can include, without limitation, injections (e.g., intravenous (IV), ID, intramuscular (IM) injection, or subcutaneous). In some cases, an antigen can be encoded by a vector (e.g., a viral vector), and the vector can be administered to a mammal.

An exemplary nucleic acid sequence encoding a human allo-MHC I can include a sequence as set forth in SEQ ID NO:1. Nucleic acid encoding a human MHC I polypeptide (e.g., an HLA-A polypeptide, an HLA-B polypeptide, or an HLA-C polypeptide) can be included within a viral vector such that cells infected with the viral vector express the encoded MHC I polypeptide. In some cases, a nucleic acid sequence

encoding a human allo-MHC I can be as described elsewhere (see, e.g., Pimtanonthai et al., 2000 *Human Immunology* 61:808-815). In some cases, a nucleic acid sequence encoding a human allo-MHC I can be as set forth in a database such as the National Center for Biotechnology Information (see, e.g., GenBank® accession numbers M84384.1, AF181842, and AF181843).

SEQ ID NO:1

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ATGCGGGTCACGGCGCCCCGAACCCTCCTCCTGCTGCTCTGGGGGGCAGTGGCCCTGACC
GAGACCTGGGCTGGCTCCCACTCCATGAGGTATTTCCACACCTCCGTGTCCCGGCCCGGC
10 CGCGGGGAGCCCCGCTTCATCACCGTGGGCTACGTGGACGACACGCTGTTCTGTGAGGTTC
GACAGCGACGCCACGAGTCCGAGGAAGGAGCCGCGGGCGCCATGGATAGAGCAGGAGGGG
CCGGAGTATTGGGACCGGGAGACACAGATCTCCAAGACCAACACACAGACTTACCGAGAG
AGCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCCGGGTCTCACATCATCCAG
AGGATGTATGGCTGCGACCTGGGGCCGGACGGGCGCCTCCTCCGCGGGCATAACCAGTAC
15 GCCTACGACGGCAAAGATTACATCGCCCTGAACGAGGACCTGAGCTCCTGGACCGCGGGC
GACACCGCGGGCTCAGATCACCCAGCGCAAGTGGGAGGCGGCCCGTGAGGCGGAGCAGCTG
AGAGCCTACCTGGAGGGCCTGTGCGTGGAGTGGCTCCGCAGACACCTGGAGAACGGGAAG
GAGACGCTGCAGCGCGCGGACCCCCCAAAGACACACGTGACCCACCACCCCATCTCTGAC
CATGAGGCCACCCTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCGGAGATCACACTGACC
20 TGGCAGCGGGATGGCGAGGACCAAACCTCAGGACACTGA

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An exemplary nucleic acid sequence encoding a human allo-MHC II can include a sequence as set forth in SEQ ID NO:2. Nucleic acid encoding a human MHC II polypeptide (e.g., an HLA-DP polypeptide, an HLA-DM polypeptide, an HLA-DOA polypeptide, an HLA-DOB polypeptide, an HLA-DQ polypeptide, or an HLA-DR polypeptide) can be included within a viral vector such that cells infected with the viral vector express the encoded MHC II polypeptide. In some cases, a nucleic acid sequence encoding a human allo-MHC II can be as described elsewhere (see, e.g., Robinson et al., 2005 *Nucleic Acids Research* 331:D523-526; and Robinson et al., 2013 *Nucleic Acids Research* 41:D1234-40).

SEQ ID NO:2

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ATGGTGTGTCTGAGGCTCCCTGGAGGCTCCTGCATGGCAGTTCTGACAGTGACACTGATG
GTGCTGAGCTCCCCACTGGCTTTGGCTGGGGACACCAGACCACGTTTCTTGAGTACTCT
35 ACGGGTGAGTGTTATTTCTTCAATGGGACGGAGCGGGTGCGGTTACTGGAGAGACACTTC
CATAACCAGGAGGAGCTCCTGCGCTTCGACAGCGACGTGGGGGAGTTCCGGGCGGTGACG
GAGCTGGGGCGGCCTGTGCGCGAGTCCTGGAACAGCCAGAAGGACATCCTGGAAGACAGG
CGCGCCGCGGTGGACACCTATTGCAGACACAACCTACGGGGCTGTGGAGAGCTTCACAGTG
CAGCGGCGAGTCCATCCTAAGGTGACTGTGTATCCTTCAAAGACCCAGCCCTGCAGCAC

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CACAACCTCCTGGTCTGTTCTGTGAGTGGTTTCTATCCAGGCAGCATTGAAGTCAGGTGG
 TTCCGGAATGGCCAGGAAGAGAAGACTGGGGTGGTGTCCACGGGCCTGATCCACAATGGA
 GACTGGACCTTCCAGACCCTGGTGATGCTGGAAACAGTTCCTCGGAGTGGAGAGGTTTAC
 ACCTGCCAAGTGGAGCACCCAAGCGTGACAAGCCCTCTCACAGTGGAATGGAGAGCACGG
 5 TCTGAATCTGCACAGAGCAAGATGCTGAGTGGAGTCGGGGGCTTTGTGCTGGGCCTGCTC
 TTCCTTGGGGCCGGGCTGTTTCATCTACTTCAGGAATCAGAAAGGACACTCTGGACTTCAG
 CCAAGAGGATTCCTGAGCTGA

In some cases, a nucleic acid set forth in Figure 17 can be included within a viral
 10 vector to express a human MHC I polypeptide, and that viral vector can be used to active
 naïve T cells within a mammal.

In some cases, a viral vector for activating naïve T cells *in vivo* as described
 herein can be designed to express a fragment of an MHC I polypeptide or a fragment of
 an MHC II polypeptide. A fragment of an MHC I polypeptide or an MHC II polypeptide
 15 can be from about 182 amino acids to about 273 amino acids (e.g., from about 182 amino
 acids to about 250 amino acids, from about 182 amino acids to about 225 amino acids,
 from about 182 amino acids to about 200 amino acids, from about 200 amino acids to
 about 273 amino acids, from about 225 amino acids to about 273 amino acids, from about
 250 amino acids to about 273 amino acids, from about 190 amino acids to about 260
 20 amino acids, from about 200 amino acids to about 250 amino acids, from about 215
 amino acids to about 235 amino acids, from about 200 amino acids to about 220 amino
 acids, from about 220 amino acids to about 240 amino acids, from about 240 amino acids
 to about 260 amino acids, or from about 260 amino acids to about 280 amino acids) in
 length.

25 A viral vector for activating naïve T cells *in vivo* as described herein can be, or can
 be derived from, a viral vaccine. In some cases, a viral vector used as described herein
 can be replication-defective. In some cases, a viral vector used as described herein can be
 immunogenic. Examples of viral vectors that can be designed to encode an MHC class I
 or class II polypeptide and used to active T cells (e.g., naïve T cells) within a mammal
 30 include, without limitation, picornavirus vaccines, adenovirus vaccines, rhabdoviruses
 (e.g., vesicular stomatitis viruses (VSV)), paramyxoviruses, and lentiviruses. In some
 cases, naïve T cells described herein can be activated *in vivo* by administering to a human
 an immunogenic, replication-defective adenoviral vector encoding an allo-MHC I. An
 exemplary adenoviral vector encoding an allo-MHC I and/or allo-MHC-class II is shown
 35 in Figure 4B.

This document also provides materials and methods for treating mammals (e.g., humans) having cancer (e.g., a cancer including cancer cells that express a tumor antigen). For example, naïve T cells described herein (e.g., naïve T cells expressing a tumor-specific antigen) can be activated *in vivo* to treat humans having cancer. In some cases, *in vivo* activation of naïve T cells as described herein can be used to reduce the number of cancer cells (e.g., cancer cells expressing a tumor antigen) within a mammal. In some cases, *in vivo* activation of naïve T cells as described herein can be used to slow and/or prevent recurrence of a cancer (e.g., a cancer in remission). In some cases, *in vivo* activation of naïve T cells as described herein can be used to target quiescent and/or non-dividing cancer cells (e.g., cancer cells expressing tumor antigens).

In some cases, the methods described herein for treating mammals (e.g., humans) having cancer can include identify the mammal as having cancer. Any appropriate method can be used to identify a mammal as having cancer. Once identified as having cancer, naïve T cells (e.g., naïve T cells obtained from the mammal having cancer) can be engineered (e.g., engineered *in vitro* or *in vivo*) to express antigen receptors (e.g., tumor-specific antigen receptors), and activated *in vivo* as described herein.

Any type of mammal having cancer can be treated using the materials and methods described herein. Examples of mammals that can be treated by *in vivo* activation of naïve T cells as described herein include, without limitation, primates (e.g., humans and monkeys), dogs, cats, horses, cows, pigs, sheep, rabbits, mice, and rats. For example, humans having cancer can be treated using *in vivo* activation of naïve T cells as described herein.

Any appropriate type of cancer can be treated using the materials and methods described herein. In some cases, a cancer to be treated as described herein can include one or more solid tumors. In some cases, a cancer to be treated as described herein can be a cancer in remission. In some cases, a cancer to be treated as described herein can include quiescent (e.g., dormant or non-dividing) cancer cells. In some cases, a cancer to be treated as described herein can be cancer that has escaped and/or has been non-responsive to chemotherapy. Examples of cancers that can be treated by *in vivo* activation of naïve T cells as described herein include, without limitation, leukemias (e.g., acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), chronic myelogenous leukemia (CML), acute monocytic leukemia (AMOL)), lymphomas (e.g., Hodgkin's

lymphomas and non-Hodgkin's lymphomas), myelomas, ovarian cancer, breast cancer, prostate cancer, colon cancer, germ cell tumors, hepatocellular carcinoma, bowel cancer, lung cancer, and melanoma (e.g., malignant melanoma).

The materials and methods described herein can be used to specifically target a
5 cell (e.g., a cancer cell) expressing an antigen (e.g., a tumor antigen such as a tumor-specific antigen). For example, *in vivo* activation of naïve T cells as described herein can include engineering the naïve T cells to express a tumor-specific antigen receptor that can target (e.g., recognize and bind to) a tumor antigen. In some cases, a tumor antigen can be a cell surface tumor antigen. Examples of tumor antigens that can be targeted by *in*
10 *vivo* activated T cells expressing a tumor-specific antigen receptor include, without limitation, MUC-1 (associated with breast cancer, multiple myeloma, colorectal cancer, and pancreatic cancer), HER-2 (associated with gastric cancer, salivary duct carcinomas, breast cancer, testicular cancer, and esophageal cancer), and ER (associated with breast cancer, ovarian cancer, colon cancer, prostate cancer, and endometrial cancer).

In cases where naïve T cells described herein (e.g., naïve T cells expressing
15 tumor-specific antigen receptors) are engineered *ex vivo* to express a heterologous antigen receptor (e.g., a heterologous tumor-specific antigen receptor) as described herein and administered (e.g., by adoptive transfer) to a mammal (e.g., a human), any appropriate method can be used to administer the naïve T cells (e.g., engineered naïve T cells).
20 Examples of methods of administering naïve T cells engineered to express a heterologous antigen receptor to a mammal can include, without limitation, injection (e.g., IV, ID, IM, or subcutaneous injection). For example, naïve T cells expressing tumor-specific antigen receptors can be administered to a human by IV injection.

In cases where naïve T cells described herein (e.g., naïve T cells expressing
25 tumor-specific antigen receptors) are engineered *ex vivo* to express a heterologous antigen receptor (e.g., a heterologous tumor-specific antigen receptor) and administered (e.g., by adoptive transfer) to a mammal (e.g., a human), any appropriate number of naïve T cells (e.g., engineered naïve T cells) can be administered to a mammal (e.g., a mammal having cancer). In some cases, from about 200 naïve T cells described herein to about 1500
30 naïve T cells described herein (e.g., from about 200 naïve T cells to about 1300 naïve T cells, from about 200 naïve T cells to about 1250 naïve T cells, from about 200 naïve T cells to about 1000 naïve T cells, from about 200 naïve T cells to about 750 naïve T cells, from about 200 naïve T cells to about 500 naïve T cells, or from about 200 naïve T cells

to about 400 naïve T cells) can be administered to a mammal (e.g., a human). For example, about 300 naïve T cells expressing tumor-specific antigen receptors can be administered to a human having cancer where the naïve T cells are then activated *in vivo* by allo-MHC I (e.g., allo-MHC I administered to the human having cancer using an immunogenic, replication-defective adenoviral vector encoding an allo-MHC I).

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Priming Cytotoxic T Cells (CTLs).

To examine if CTLs could be primed to hunt and kill quiescent cells expressing targetable antigens, 1200 OT-1 T cells were adoptively transferred into RIP-OVA mice (expressing the ovalbumin (OVA) antigen in pancreatic islets), and then activated with TMEV-OVA picornavirus vaccine.

Pancreatic tissues were examined at using H&E staining and IHC staining for insulin. Pancreatic inflammation was seen within 5 days of CTL induction by virus (Figure 2A). Significant destruction of islets was observed in surviving mice on day 21 (Figure 2B). No virus was detected in the pancreas by PCR. As few as 300 naïve T cells activated *in vivo* by a picornavirus vaccine elicited complete destruction of normal virus free pancreatic islets within 10 days of activation. In contrast, 8×10^7 OT-1 spleen cells activated in donor mice and transferred into RIP-OVA mice were not pathogenic.

These results demonstrate that activated T cells can scan cells in the body for relevant antigens and elicit immune destruction in the absence of preexisting inflammation.

Example 2: Activation of Allo-Reactive Cytotoxic T Cells (CTLs).

To determine whether adenovirus encoding allogeneic MHC I molecules can activate allo-reactive CTL, the allogeneic MHC class I gene was expressed in the context of an adenovirus infection into LN antigen presenting cells.

Adenovirus expressing Cre recombinase were introduced into the lymphatics of mTmG-reporter mice by intradermal injection. mTmG-reporter mice express a floxed membrane red fluorescent “tomato” and a silenced membrane GFP gene. In the presence of expressed cre, tomato is silenced and GFP is activated. Tomato expressing and GFP

expressing T cells can be distinguished by fluorescent microscopy following introduction of an adenovirus expressing cre or a control adenovirus. Successful transduction results in the transition from red to green fluorescence. Cre recombinase was transduced in sub capsular sinus macrophage (Figures 3A – 3C).

5 A replication-defective adenovirus (serotype 6) vector expressing a mutant MHC molecule which functions as a universal alloantigen (Figure 4A) was introduced into LN by intradermal injection. Four days after introduction of adenovirus encoding allogeneic MHC I, syngeneic (BALB/c), allogeneic (B6), and third party (C3H) labeled target cells were adoptively transferred IV into challenged hosts in an *in vivo* CTL assay. Four hours
10 later spleen cells were harvested and analyzed by flow cytometry for the presence of introduced target cells. B6 target cells (targets expressing introduced allo-MHC I) were completely eliminated *in vivo*. Potent allo-reactive CD8⁺ T cells were activated in just 4 days (Figure 5).

These results demonstrate that intradermally injected adenovirus expressing allo-MHC I can present allo-MHC I antigen in sub capsular sinus macrophages and can
15 activate CTLs that target cells expressing allo-MHC I.

Example 3: Adoptive Transfer of Naïve Cytotoxic T Cells (CTLs).

To examine if adoptively transferred naïve CTL precursors migrate to secondary lymphoid organs and become activated by adeno-MHCI virus, allotype-marked naïve T
20 cells were labeled with CFSE and adoptively transferred intravenously into naïve hosts which were subsequently challenged intradermally with adeno-MHCI to elicit an allo-reactive CTL response from the transferred cells.

Adoptively transferred CFSE-labeled T cells migrated to the LN where they encountered and responded to transduced alloMHCI molecules (Figures 6A and 6C).
25 Stimulated cells proliferated when stimulated with allo-MHCI, diluting the CFSE (Figure 6C). The CFSE-dilute population displayed a more activated phenotype expressing high CD44 and PD-1 (Figures 6D) relative to the CFSE-dilute cells isolated from lymph nodes challenged with control adenovirus (Figures 6B). Approximately 4.5% of the transferred cells present on day 4 had proliferated (Figures 6A and 6C) and exhibited upregulation of
30 activation markers (Figures 6B and 6D).

These results demonstrate that adoptively transferred CD8⁺ T cells can migrate to the LN and can be activated by an alloMHC I encoding adenovirus vaccine.

Example 4: In Vivo Activation of Naïve Cytotoxic T Cells (CTLs).

To examine whether *in vivo* activated T cells can migrate into tumors, an approach for evaluating the efficiency of viral transduction of T cells *ex vivo* was established using mTmG-reporter mice. Naïve CD8⁺ spleen cells from MTMG-reporter mice were
 5 transduced with lentivirus expressing cre by centrifugal concentration of virus and polybrene, and were subsequently activated with anti-CD3/CD28 + IL-2 to maintain viability in culture for 4 days. Successful transduction results in the transition from red to green fluorescence (Figure 7 and Figure 8). Using the MTMG-reporter mouse, the efficiency of transfection, the migration of adoptively transferred T cells into lymph
 10 nodes, and migration of the adoptively transferred T cells into tumors can be determined.

Example 5: In Vivo Activation of Naïve Cytotoxic T Cells (CTLs).

Humanized NSG (hu-NSG) mice with established human hematopoiesis provide a model for using lentivirus CAR to establish proof of concept. hu-NSG mice in donor matched batches with verified human leukocytes in circulation were obtained. These
 15 mice were used as donors of human cells for a CAR transduction scheme.

To determine if a CAR could activate CTLs *in vivo*, freshly isolated T cells were transduced with a lentiviral vector expressing human CAR19 (lenti-CAR19). Pooled CD4 & CD8 T cells transduced with lenti-CAR19 prior to or after activation (anti-CD3/CD28) and cultured 4 days to allow gene expression, then stained with anti-mouse
 20 antibody and analyzed using flow cytometry. Freshly isolated spleen cells were transduced with lenti-CAR19 for 1 hour and immediately transferred into syngeneic hu-NSG recipients (1 donor spleen/recipient). Mice also received Ad6-K^b vaccine at the time of cell transfer. Approximately 10% of the recovered human spleen cells were CAR⁺ in the three recipients. As shown in Figure 9, human T cells were effectively transduced
 25 with lenti-CAR19, but mouse cells were not.

Hu-NSG mice lack lymph nodes. The absence of lymph nodes in hu-NSG mice required a change in approach. To evaluate the effectiveness of intradermal introduction of non-replicating virus in hu-NSG mice, Evans blue was injected intradermally in the tail to mark inguinal lymph node in WT, NOD Scid IL-2R $\gamma^{-/-}$ (NSG), and human CD34⁺
 30 hematopoietic cell reconstituted NSG mice (hu-NSG) (Figure 10).

To determine if alternative routes of administration could be used for *in vivo* CTL, replication-defective adenoviral vectors encoding an allo-MHC I (Ad6-alloMHC (K^b)) were delivered to hu-NSG mice multiple routes, and the ability to induce strong CTL

activity was assessed. BALB/c mice received 10^{10} Ad6-K^b IV, ID, or IP. 1 week later, the mice received differentially labeled BALB/c (self) and B6 (alloMHC) target cells IV. Cells migrating into the spleen were assessed for both introduced populations. Effectiveness was indicated by the relative depletion of the B6 target cells. IV, ID, and IP vaccination were equally effective for inducing strong CTL activity (Figure 11). In a subsequent experiment, mice received half the vaccine IV and half IP as the distribution and trafficking of human immune cells in the spleens and peritoneum of NSG mice is poorly defined.

To determine whether adenovirus encoding allogeneic MHC I molecules can activate allo-reactive CTL to eliminated cells expressing a target antigen, hu-NSG mice were administered lentivirus-CAR19 transduced hu-NSG spleen cells and replication defective adenoviruses encoding the MHC allogeneic antigen H-2K^b. An overview of the method is shown in Figure 12. Three hu-NSG mice with known T cell reconstitution were selected as lymphoid donors. The human leukocyte composition of hu-NSG mice selected as donors and hu-NSG mice selected as recipients are shown in Figure 13. Spleen cells from donor animals were recovered, pooled, red cells lysed using ACK and then the whole product was suspended in 100 μ L of undiluted lenti-CAR19 virus (MOI). Polybrene was added for final concentration of 8 μ g/mL. The suspension was centrifuged at 800 x g for 90 minutes at 31 °C. The viral supernatant was removed, and the cell pellet was suspended in 300 μ L PBS and injected IV (100 μ L/mouse). 5×10^9 viral particles of replication defective adenovirus 6 encoding the MHC allogeneic antigen H-2K^b was injected IV, and an identical dose was injected IP. The mice were monitored daily with no detrimental phenotypes observed for one week. On day 7, the mice were bled, and the composition of human B cells in the blood was assessed.

To determine if *in vivo* CTL induced anti-K^b cytotoxic activity in hu-NSG hosts, mice were challenged with a mixture of K^b- and K^b+ target cells, and spleens of the recipient mice were examined. Four hours prior to harvesting blood and spleen cells from the hu-NSG recipients (which had received lentivirus-CAR19 transduced hu-NSG spleen cells and Ad6-H-2K^b vaccine 1 week earlier), mice were challenged with a 1:1 mixture of K^b- syngeneic NOD splenic target cells and K^b+ allogeneic B6 splenic target cells differentially labeled with CFSE. Following the 4 hour *in vivo* incubation period, each of the spleens of the recipient mice was examined for the ratio of persisting labeled K^b- and K^b+ target cells. The expected 1:1 ratio was altered in all three recipients indicating a

preferential killing of the K^{b+} spleen cells (Figure 14, panel A). The ratio of recovered K^{b+} cells was significantly lower relative to the K^{b-} target cells (Figure 14, panel B). This analysis indicated CTL activity was induced by vaccination with Ad6-H-2K^b in the hu-NSG mice targeting K^b expressing cells.

5 To determine if *in vivo* CTL activated against cells expressing a target antigen, human spleen cells from hu-NSG hosts that received CART treatment and AD6 vaccination were assessed for expression of the CAR19 protein. Raw data of the drop in B cell numbers in hu-NSG mice is shown in Figure 15. The absolute numbers of recovered B cells pre and post therapy were highly significant. However, two variables
10 could have contributed to this conclusion, non-specific depletion of peripheral blood cell populations, including B cells, by repeated blood sampling, and the intended effects of CAR T cell therapy. To confirm the drop in B cell numbers was due to CAR T cell therapy, data was normalized to remove possible non-specific depletion effects. The normalization of the post treatment values to the pretreatment values using the formula
15 (total CD45⁺ cell counts pretreatment/CD45 cell counts post treatment X absolute counts of cell lineage⁺ cells post treatment) was a conservative approach, reducing the magnitude of observed differences between pre and post treatment values in the B cell compartment to account for non-specific depletion of B cells by repetitive sampling of the blood. One-tailed hypothesis testing used a paired T Test to reflect the hypothesis. The
20 apparent increase in T cells following therapy is consistent with previous CART therapy findings. However, evaluation of this possibility was not part of the original hypothesis, therefore, a two tailed test was applied. The absence of change in myeloid counts suggests the observed drop in B cells and the apparent rise of T cells appears to be cell lineage specific (Figure 16). There appears to be a correlation between the degree of B
25 cell depletion and rise in T cells and in the level of CAR19 expression (Figure 16, panel B) in the spleens of the recipient mice, associations also seen previously in CART therapy.

This analysis verified Ad6-K^b activated antigen-specific killing. The mice demonstrated activity against the CD19 target after administration of Ad6-MHC, as
30 demonstrated by the depletion of circulating CD19⁺ B cells in the recipient mice.

These results demonstrate that naïve T cells expressing tumor-specific antigen receptors can be specifically activated (e.g., to become CTLs) *in vivo* by encountering a target antigen, and the *in vivo* activated T cells can target cells expressing the antigen.

Example 6: Generation of Viral Vectors.

To develop a viral vector encoding rare HLA class I molecules such as HLA-B*4028, partial nucleic acid sequences encoding exons 2 and 3 were obtained from publically available database (see, e.g., GenBank: AF181842 and AF181843, respectively; since replaced with AH008245.2) and were used to guide modification of the full-length coding sequence for HLA-B*4004 (see, e.g., GenBank: M84384.1) capable of producing a full-length HLA-B*4028 polypeptide (e.g., SEQ ID NO:3). To develop a viral vector also encoding rare HLA class II molecules such as HLA-DRB1*12:01:01:01, SEQ ID NO:2 was obtained from publically available data base (see, e.g., Robinson et al., 2005 *Nucleic Acids Research* 331:D523-526; and Robinson et al., 2013 *Nucleic Acids Research* 41:D1234-40), and used to produce a full-length HLA-DRB1*12:01:01:01 polypeptide (e.g., SEQ ID NO:4).

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for activating a naïve T cell in a mammal, said method comprising:
 - (a) engineering a naïve T cell to express an antigen receptor, thereby forming an engineered naïve T cell; and
 - (b) activating said engineered naïve T cell in said mammal.
2. The method of claim 1, wherein said mammal is a human.
3. The method of any one of claims 1-2, wherein said naïve T cell is a naïve cytotoxic T lymphocyte.
4. The method of any one of claims 1-3, wherein said antigen receptor is a chimeric antigen receptor.
5. The method of any one of claims 1-4, wherein said antigen receptor is a tumor-specific antigen receptor.
6. The method of any one of claims 1-5, wherein said engineering comprises *ex vivo* engineering.
7. The method of claim 6, wherein said *ex vivo* engineering comprises:
 - (a) obtaining said naïve T cell from said mammal;
 - (b) introducing nucleic acid encoding said antigen receptor into said naïve T cells to produce said engineered naïve T cell; and
 - (b) administering said engineered naïve T cells to said mammal.
8. The method of claim 7, wherein said introducing comprises transducing said naïve T cells with a viral vector encoding said antigen receptor.
9. The method of claim 8, wherein said viral vector is a lentiviral vector or a retroviral vector.

10. The method of any one of claims 7-9, wherein said administering comprises intravenous injection.
11. The method of any one of claims 1-11, wherein said engineering comprises *in situ* engineering.
12. The method of claim 11, wherein said *in situ* engineering comprises administering a viral vector encoding said antigen receptor to said mammal.
13. The method of claim 12, wherein said administering comprises intradermal injection.
14. The method of claim 13, wherein said intradermal injection is directly into a lymph node of said mammal.
15. The method of any one of claims 12-14, wherein said viral vector is an adenoviral vector.
16. The method of any one of claims 1-15, wherein said activating said engineered naïve T cell *in vivo* comprises administering a viral vector encoding an antigen to said mammal.
17. The method of claim 16, wherein said antigen is an alloantigen.
18. The method of claim 17, wherein said alloantigen is an allogeneic major histocompatibility complex class I antigen.
19. The method of any one of claims 16-18, wherein said viral vector is an adenoviral vector.
20. The method of claim 19, wherein said administering comprises intradermal injection.

21. The method of claim 20, wherein said intradermal injection is directly into a lymph node of said mammal.
22. A method for treating a mammal having cancer, said method comprising:
(a) engineering a naïve T cell to express a tumor-specific antigen receptor, thereby forming an engineered naïve T cell; and
(b) activating said engineered naïve T cell *in vivo*.
23. The method of claim 22, wherein said mammal is a human.
24. The method of any one of claims 22-23, wherein said cancer is selected from the group consisting of acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), chronic myelogenous leukemia (CML), acute monocytic leukemia (AMOL)), Hodgkin's lymphoma, non-Hodgkin's lymphoma, myelomas, ovarian cancer, breast cancer, prostate cancer, and colon cancer.
25. The method of any one of claims 22-24, wherein said cancer comprises cancer cells expressing a tumor-specific antigen.
26. The method of claim 25, wherein said naïve T cell is engineered to express a tumor-specific antigen receptor that targets said tumor-specific antigen.
27. The method of claim 26, wherein said tumor-specific antigen is selected from the group consisting of mucin 1 (MUC-1), human epidermal growth factor receptor 2 (HER-2), and estrogen receptor (ER).
28. The method of any one of claims 22-27, wherein said engineering comprises *ex vivo* engineering.
29. The method of claim 28, wherein said *ex vivo* engineering comprises:

- (a) obtaining said naïve T cells from said mammal;
- (b) introducing nucleic acid encoding said antigen receptor into said naïve T cells to produce said engineered naïve T cell; and
- (c) administering said engineered naïve T cells to said mammal.

30. The method of claim 29, wherein said introducing comprises transducing said naïve T cells with a viral vector encoding said antigen receptor.

31. The method of claim 30, wherein said viral vector is a lentiviral vector or a retroviral vector.

32. The method of any one of claims 29-31, wherein said administering comprises intravenous injection.

33. The method of any one of claims 29-32, wherein said administering comprises administering from about 200 to about 1500 engineered naïve T cells to said mammal.

34. The method of claim 33, wherein said administering comprises administering about 300 engineered naïve T cells to said mammal.

35. The method of any one of claims 22-34, wherein said engineering comprises *in situ* engineering.

36. The method of claim 35, wherein said *in situ* engineering comprises administering a viral vector encoding said antigen receptor to said mammal.

37. The method of claim 36, wherein said administering comprises intradermal injection.

38. The method of claim 37, wherein said intradermal injection is directly into a lymph node of said mammal.

39. The method of any one of claims 36-38, wherein said viral vector is a lentiviral vector or a retroviral vector.
40. The method of any one of claims 22-39, wherein said activating said engineered naïve T cell *in vivo* comprises administering a viral vector encoding an antigen to said mammal.
41. The method of claim 40, wherein said antigen is an alloantigen.
42. The method of claim 41, wherein said alloantigen is an allogeneic major histocompatibility complex class I antigen.
43. The method of any one of claims 40-42, wherein said viral vector is an adenoviral vector.
44. The method of claim 40, wherein said administering comprises intradermal injection.
45. The method of claim 44, wherein said intradermal injection is directly into a lymph node of said mammal.
46. The method of any one of claims 22-45, wherein said cancer comprises a solid tumor.
47. The method of any one of claims 22-46, wherein said cancer is in remission.
48. The method of any one of claims 22-47, wherein said cancer comprises quiescent cancer cells.
49. The method of any one of claims 22-48, wherein said cancer comprises cancer cells that escaped chemotherapy or are non-responsive to chemotherapy.

50. A method for obtaining an activated T cell within a mammal, wherein said activated T cell comprises a heterologous antigen receptor, wherein said method comprises:

(a) introducing nucleic acid encoding said heterologous antigen receptor into T cells obtained from a mammal *in vitro* to obtain engineered T cells,

(b) administering said engineered T cells to said mammal, and

(c) administering a virus comprising nucleic acid encoding an MHC class I polypeptide to said mammal, wherein an engineered T cell of said engineered T cells administered to said mammal in step (b) is activated.

51. The method of claim 50, wherein said mammal is a human.

52. The method of any one of claims 50-51, wherein said T cells obtained from said mammal are naïve T cells.

53. The method of claim 52, wherein said naïve T cells are naïve cytotoxic T lymphocytes.

54. The method of any one of claims 50-53, wherein said antigen receptor is a chimeric antigen receptor.

55. The method of any one of claims 50-54, wherein said antigen receptor is a tumor-specific antigen receptor.

56. The method of any one of claims 50-55, wherein said nucleic acid encoding said heterologous antigen receptor is introduced into said T cells with a viral vector comprising said nucleic acid.

57. The method of claim 56, wherein said viral vector is a lentiviral vector.

58. The method of any one of claims 50-57, wherein said engineered T cells are administered to said mammal via intravenous injection.

59. The method of any one of claims 50-58, wherein said engineered T cells are administered to said mammal via injection into a lymph node of said mammal.
60. The method of any one of claims 50-59, wherein said virus is an adenovirus or a rhabdovirus.
61. The method of any one of claims 50-60, wherein said virus is administered to said mammal via intradermal injection.
62. The method of any one of claims 50-60, wherein said virus is administered to said mammal via direct administration into a lymph node of said mammal.
63. The method of any one of claims 50-62, wherein said MHC class I polypeptide is an allogeneic MHC class I polypeptide.
64. The method of any one of claims 50-63, wherein said MHC class I polypeptide is an HLA-A, HLA-B, or HLA-C polypeptide.
65. The method of any one of claims 50-64, wherein said engineered T cell activated within said mammal in said step (c) comprises a native T cell receptor.
66. The method of any one of claims 50-65, wherein said step (c) activates a plurality of engineered T cells within said mammal.
67. The method of claim 66, wherein activated T cells of said plurality of engineered T cells comprise different native T cell receptors.
68. A method for obtaining an activated T cell within a mammal, wherein said activated T cell comprises a heterologous antigen receptor, wherein said method comprises administering, to a mammal, (a) nucleic acid encoding said heterologous antigen receptor and (b) a virus comprising nucleic acid encoding an MHC class I

polypeptide, wherein said nucleic acid is introduced into T cells within said mammal to form engineered T cells comprising said heterologous antigen receptor, wherein administration of said virus activated T cells within said mammal, and wherein at least one T cell within said mammal comprises said heterologous antigen receptor and is activated.

69. The method of claim 68, wherein said mammal is a human.

70. The method of any one of claims 68-69, wherein said at least one T is a cytotoxic T lymphocyte.

71. The method of any one of claims 68-70, wherein said antigen receptor is a chimeric antigen receptor.

72. The method of any one of claims 68-71, wherein said antigen receptor is a tumor-specific antigen receptor.

73. The method of any one of claims 68-72, wherein said nucleic acid encoding said heterologous antigen receptor is introduced into said T cells with a viral vector comprising said nucleic acid.

74. The method of claim 73, wherein said viral vector is a lentiviral vector or retroviral vector.

75. The method of any one of claims 68-74, wherein said nucleic acid is administered to said mammal via intravenous injection.

76. The method of any one of claims 68-74, wherein said nucleic acid is administered to said mammal via injection into a lymph node of said mammal.

77. The method of any one of claims 68-76, wherein said virus is an adenovirus or a rhabdovirus.

78. The method of any one of claims 68-77, wherein said virus is administered to said mammal via intradermal injection.

79. The method of any one of claims 68-77, wherein said virus is administered to said mammal via direct administration into a lymph node of said mammal.

80. The method of any one of claims 68-79, wherein said nucleic acid is administered to said mammal before said virus is administered to said mammal.

81. The method of claim 80, wherein said nucleic acid encoding said heterologous antigen receptor is introduced into said T cells with a lentiviral vector comprising said nucleic acid.

82. The method of any one of claims 68-79, wherein said nucleic acid is administered to said mammal after said virus is administered to said mammal.

83. The method of claim 82, wherein said nucleic acid encoding said heterologous antigen receptor is introduced into said T cells with a retroviral vector comprising said nucleic acid.

84. The method of any one of claims 68-83, wherein said MHC class I polypeptide is an allogeneic MHC class I polypeptide.

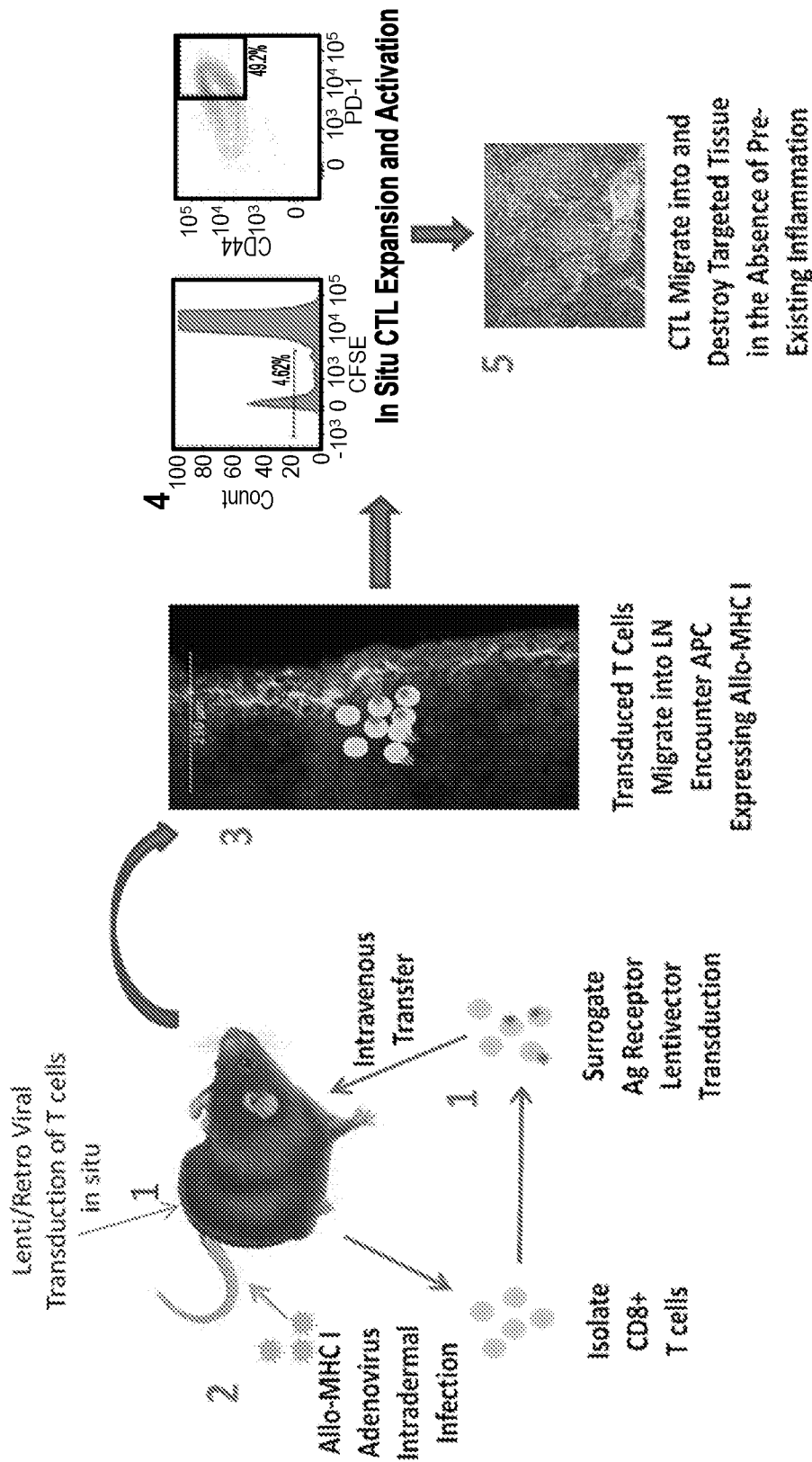
85. The method of any one of claims 68-84, wherein said MHC class I polypeptide is an HLA-A, HLA-B, or HLA-C polypeptide.

86. The method of any one of claims 68-85, wherein said at least one T cell comprises a native T cell receptor.

87. The method of any one of claims 68-86, wherein said at least one T cell is a plurality of activated T cells comprising said heterologous antigen receptor.

88. The method of claim 87, wherein each of said activated T cells of said plurality of said activated T cells comprises different native T cell receptor.
89. An isolated virus comprising nucleic acid encoding an MHC class I polypeptide.
90. The virus of claim 89, wherein said virus is a picornavirus, an adenovirus or a rhabdovirus.
91. The virus of any one of claims 89-90, wherein said virus is replication-defective.
92. The virus of any one of claims 89-91, wherein said MHC class I polypeptide is a human MHC class I polypeptide.
93. The virus of any one of claims 89-92, wherein said MHC class I polypeptide comprising the amino acid sequence set forth in SEQ ID NO:4.
94. A kit comprising a first container comprising a first virus comprising nucleic acid encoding an antigen receptor and a second container comprising a second virus according to any one of claims 89-93.
95. The kit of claim 94, wherein said first virus is a lentivirus or a retrovirus.
96. The kit of any one of claims 94-95, wherein said antigen receptor is a chimeric antigen receptor.

FIG. 1



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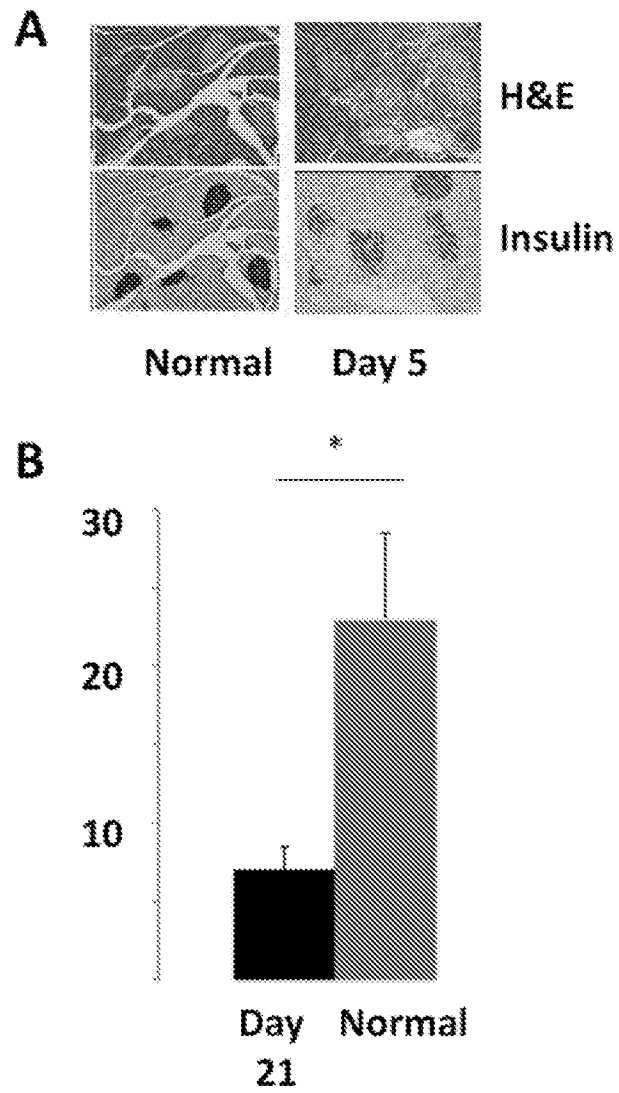


FIG. 2

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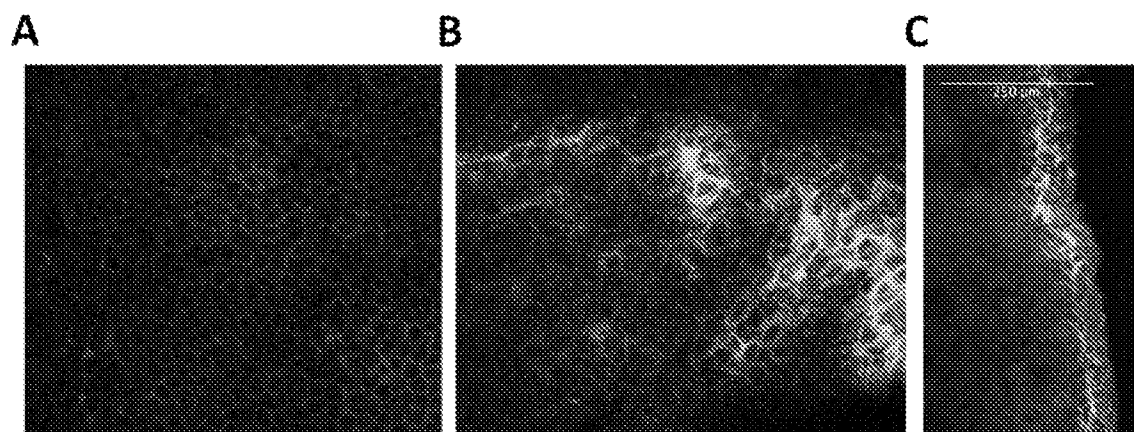


FIG. 3

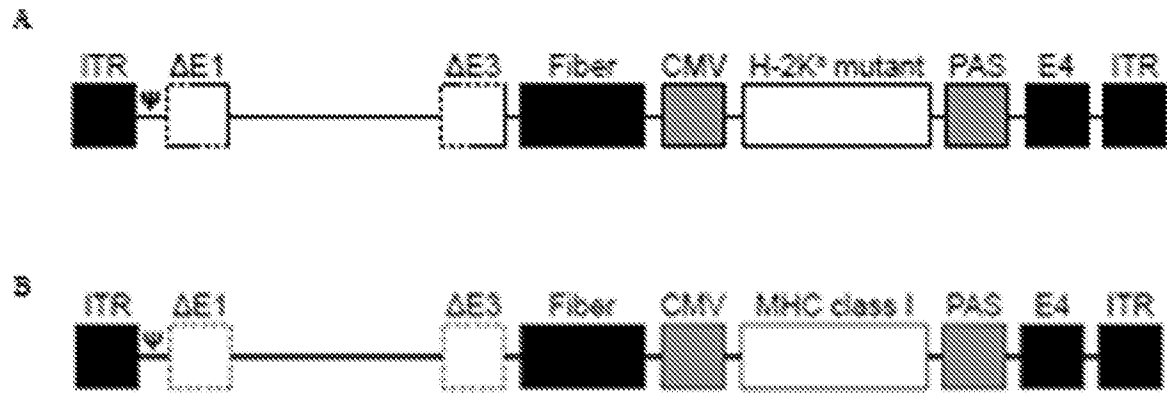


FIG. 4

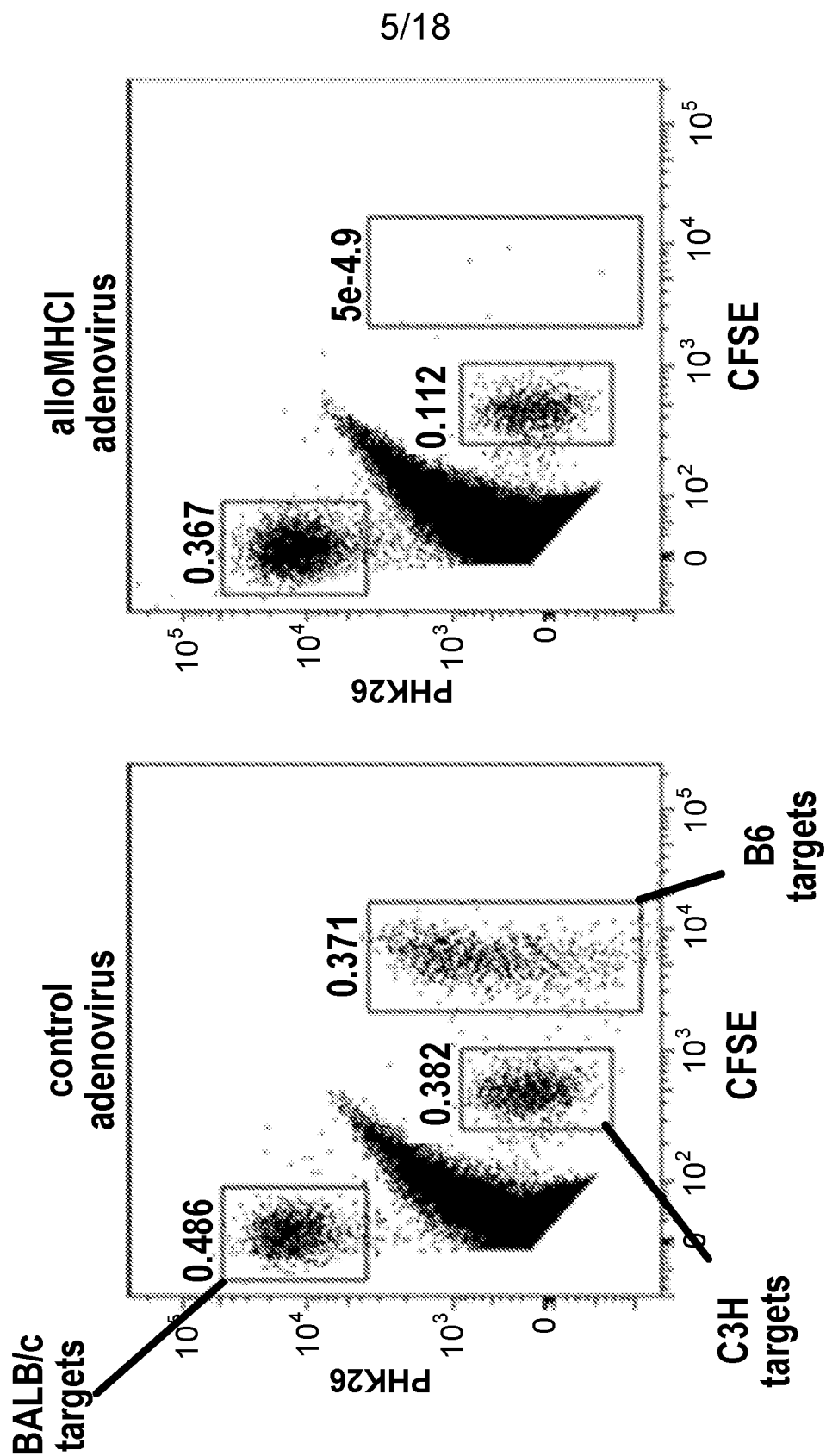


FIG. 5

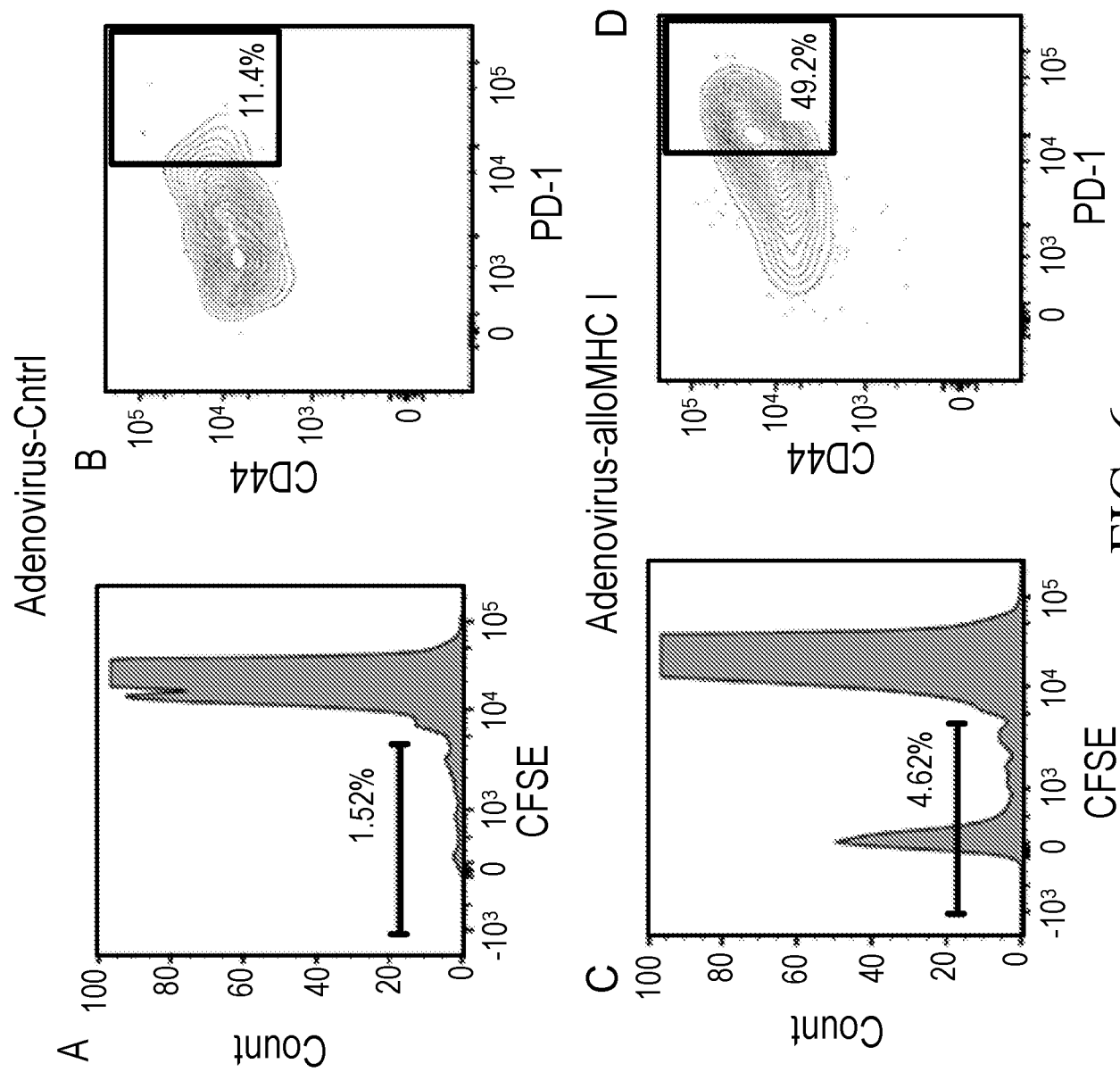


FIG. 6

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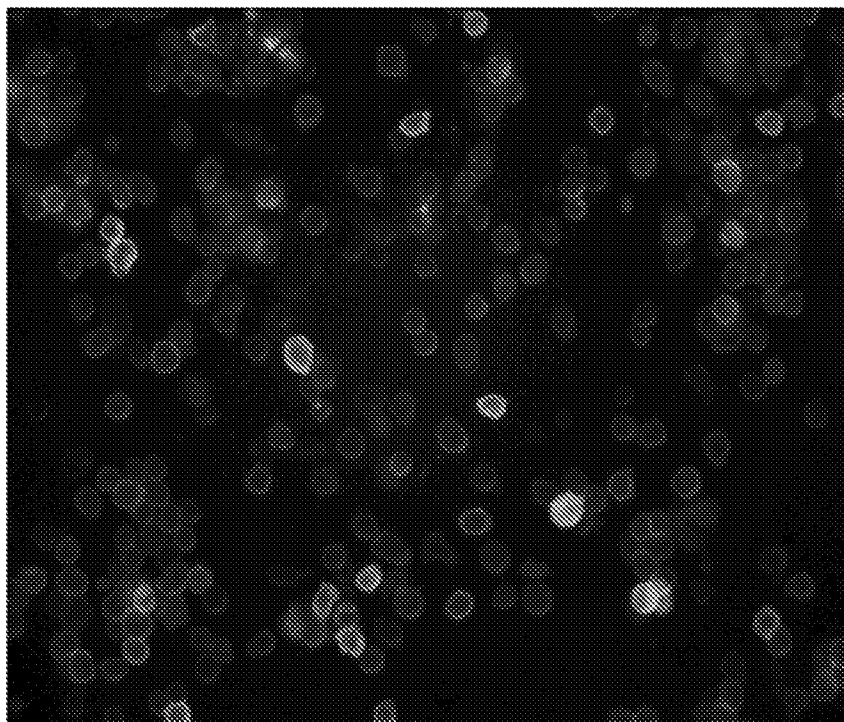


FIG. 7

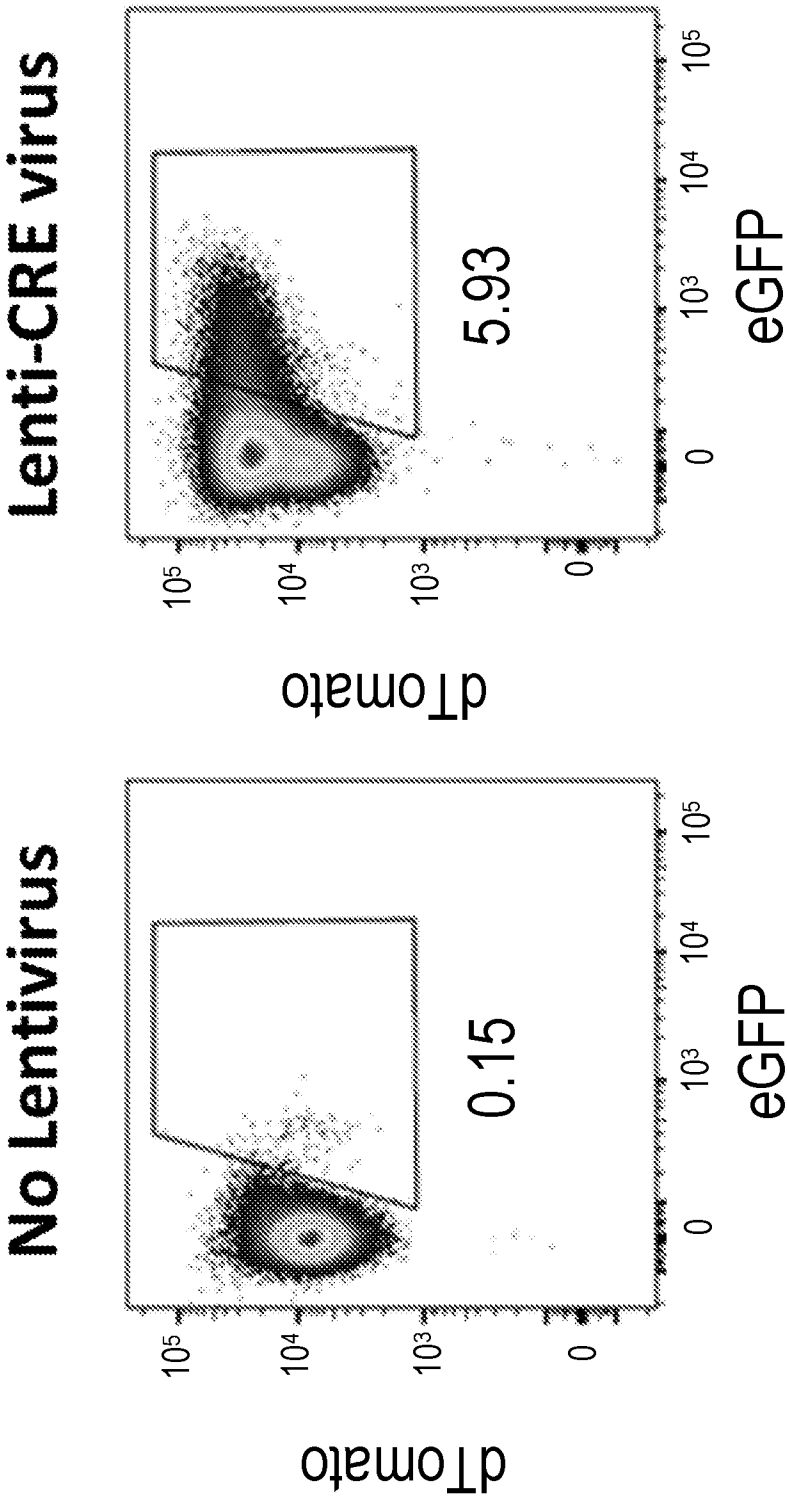


FIG. 8

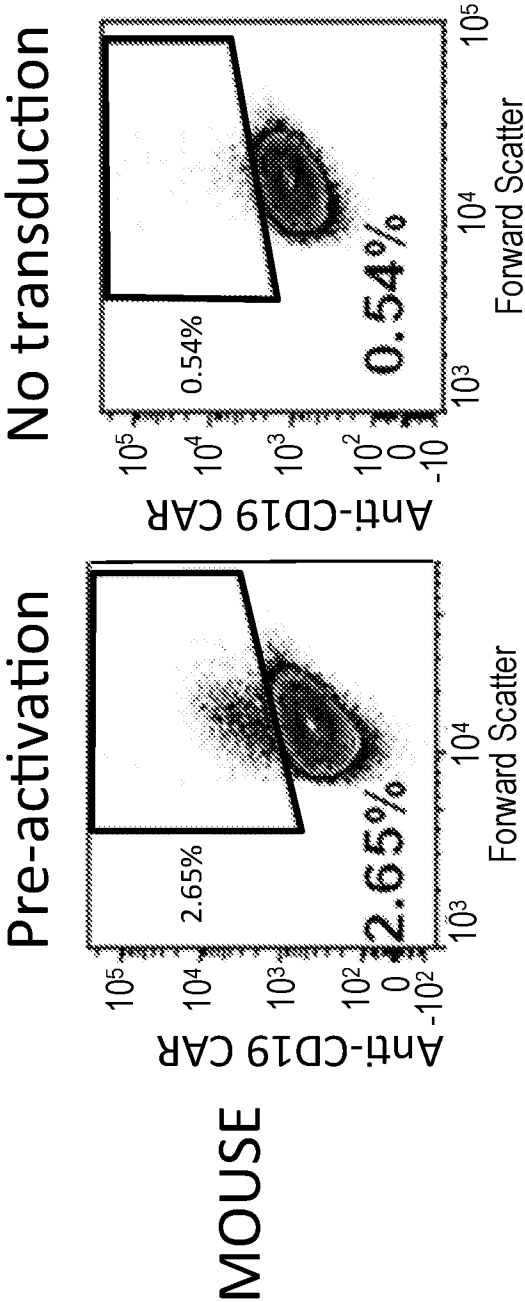
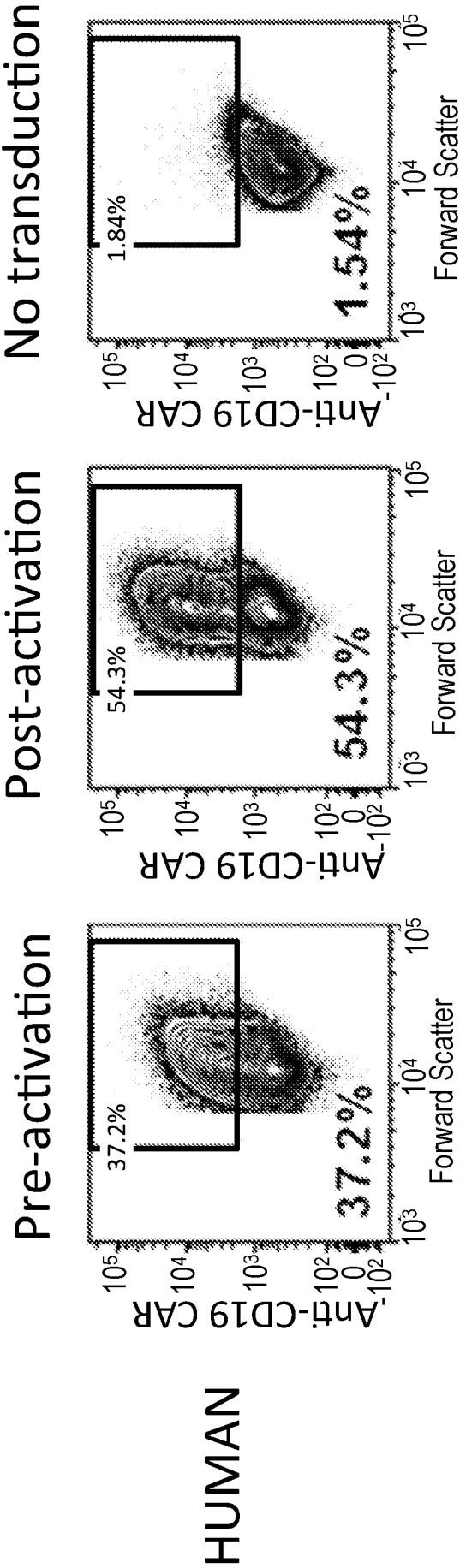


FIG. 9

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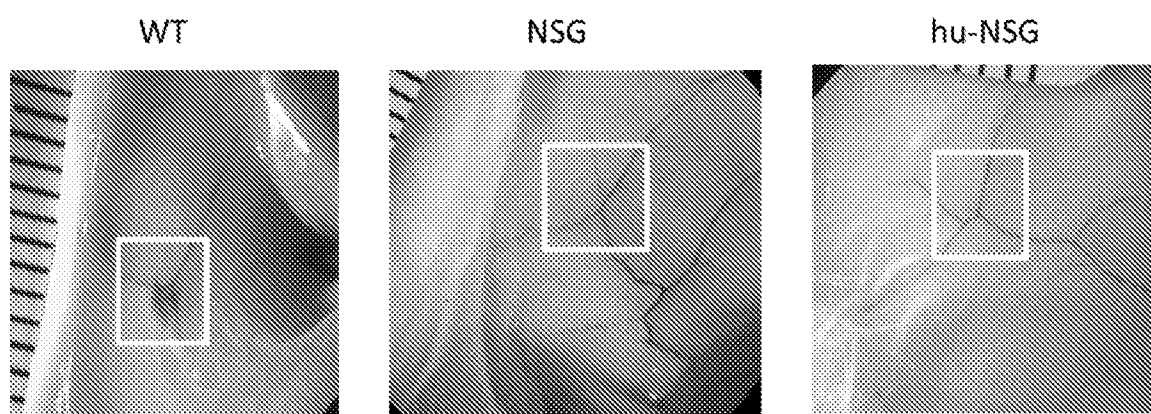


FIG. 10

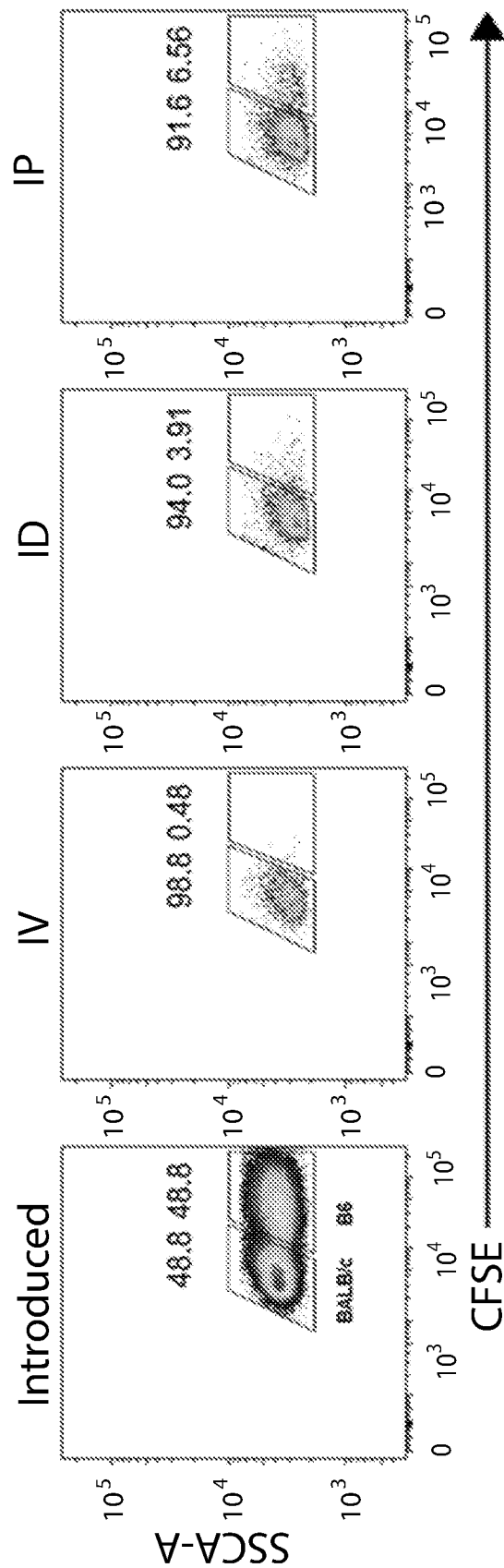


FIG. 11

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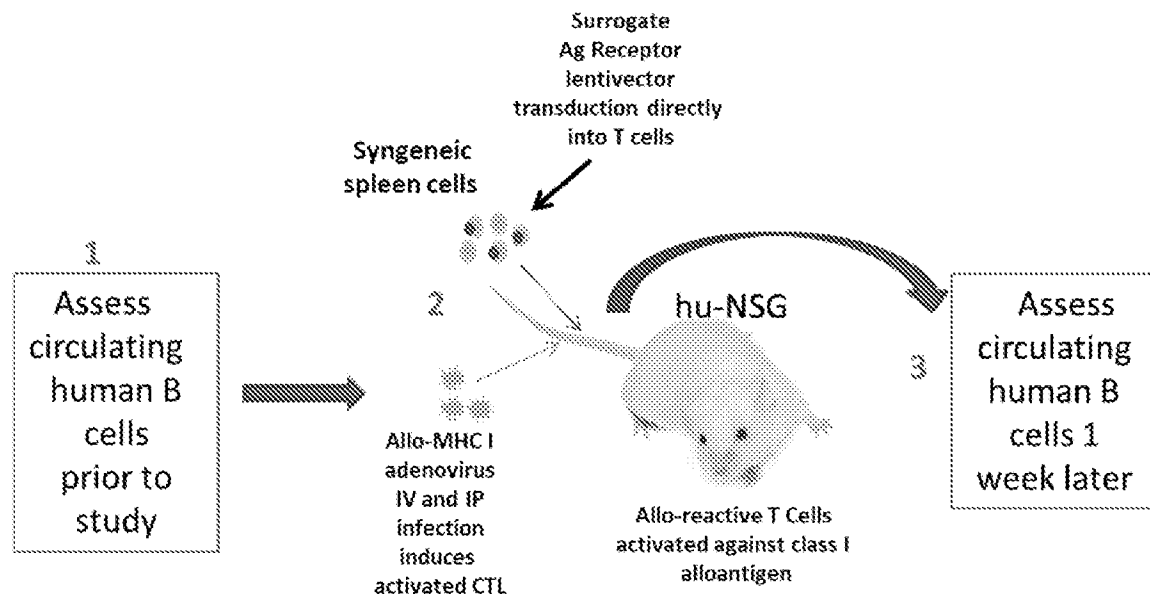


FIG. 12

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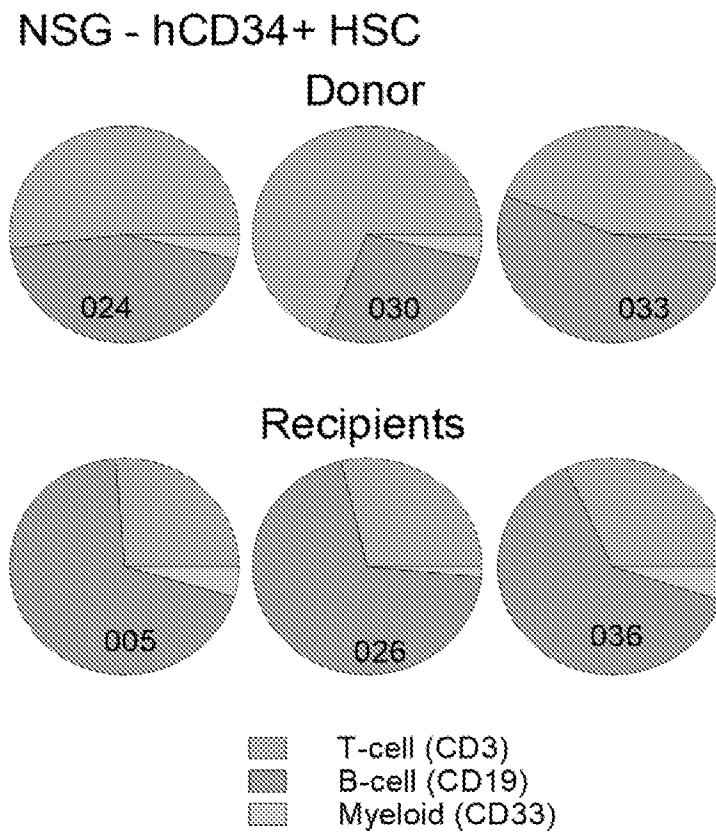


FIG. 13

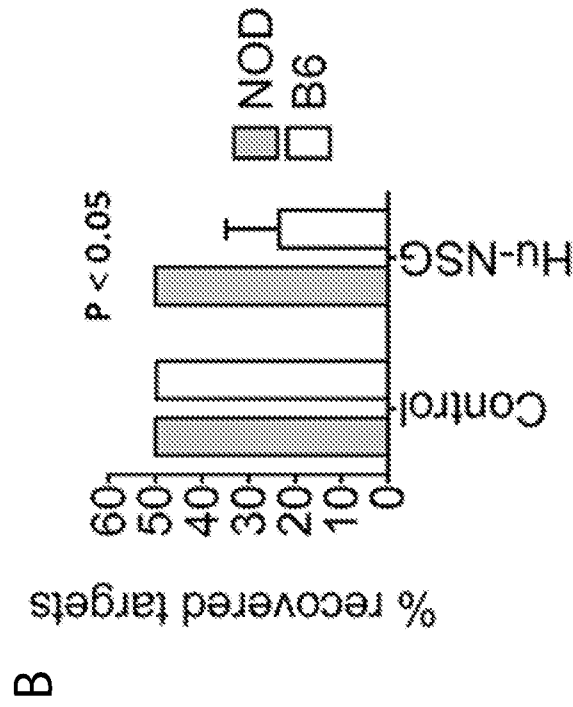
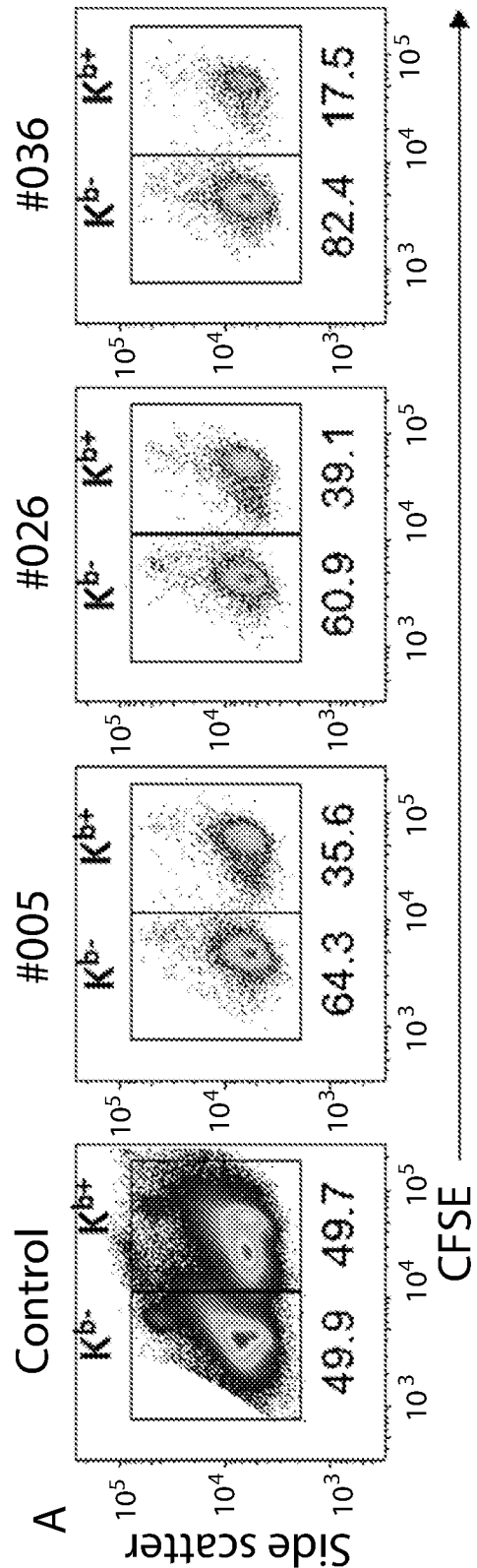


FIG. 14

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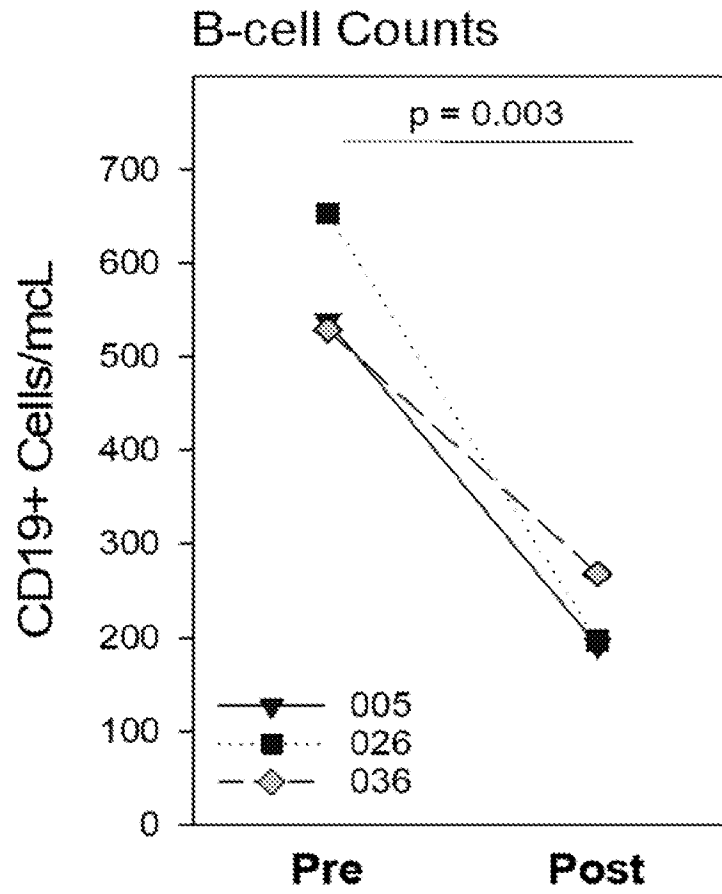


FIG. 15

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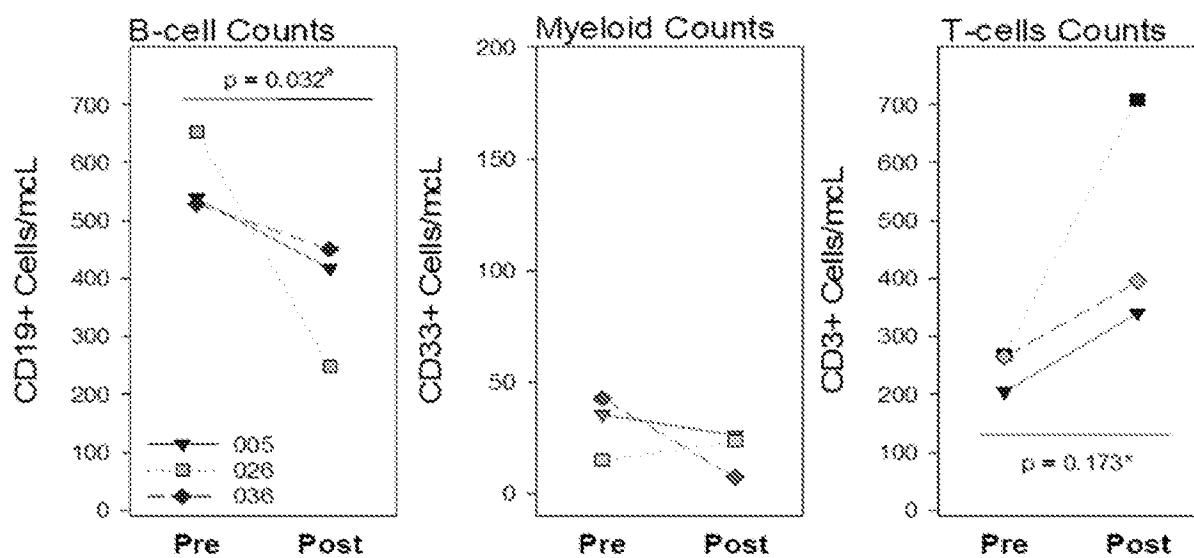


FIG. 16

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SEQ_ID_NO:1**Nucleic acid sequence of HLA-B*4028**

ATGCGGGTCACGGCGCCCCGAACCCTCCTCCTGCTGCTCTGGGGGGCAGTGGCCCTGAC
CGAGACCTGGGCTGGCTCCCACTCCATGAGGTATTTCCACACCTCCGTGTCCCGGCCCCG
GCCGCGGGGAGCCCCGCTTCATCACCGTGGGCTACGTGGACGACACGCTGTTTCGTGAGG
TTCGACAGCGACGCCACGAGTCCGAGGAAGGAGCCGCGGGCGCCATGGATAGAGCAGGA
GGGGCCGGAGTATTGGGACCGGGAGACACAGATCTCCAAGACCAACACACAGACTTACC
GAGAGAGCCTGCGGAACCTGCGCGGCTACTACAACCAGAGCGAGGCCGGGTCTCACATC
ATCCAGAGGATGTATGGCTGCGACCTGGGGCCGGACGGGCGCCTCCTCCGCGGGCATAA
CCAGTACGCCTACGACGGCAAAGATTACATCGCCCTGAACGAGGACCTGAGCTCCTGGA
CCGCGGCGGACACCGCGGCTCAGATCACCCAGCGCAAGTGGGAGGCGGCCCCGTGAGGCG
GAGCAGCTGAGAGCCTACCTGGAGGGCCTGTGCGTGGAGTGGCTCCGCAGACACCTGGA
GAACGGGAAGGAGACGCTGCAGCGCGCGGACCCCCCAAAGACACACGTGACCCACCACC
CCATCTCTGACCATGAGGCCACCCTGAGGTGCTGGGCCCTGGGCTTCTACCCTGCGGAG
ATCACACTGACCTGGCAGCGGGATGGCGAGGACCAAACTCAGGACACTGA

SEQ_ID_NO:3**Polypeptide sequence of HLA-B*4028**

MRVTAPRTLILLLLWGAVALTETWAGSHSMRYFHTSVSRPGRGEPRFITVGYVDDTLFVR
FDSDATSPRKEPRAPWIEQEGPEYWDRETQISKNTNTQTYRESLRNLRGYYNQSEAGSHI
IQRMYGCDLGPDGRLLRGHNQYAYDGKDYIALNEDLSSWTAADTAAQITQRKWEAAREA
EQLRAYLEGLCVEWLRRLHLENGKETLQRADPPKTHVTHHPISDHEATLRCWALGFYPAE
ITLTWQRDGEDQTQDTELVEVTRPAGDRTFQKWAAVVVP SGEEQRYTCHVQHEGLPKPLT
LRWEPSSQSTVP IVGIVAGLAVLAVVVIGAVVAAMCRRKSSGGKGGSYSAACSDSAQ
GSDVSLTA

FIG. 17

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SEQ_ID_NO:2**Nucleic acid sequence of HLA-DRB1*12:01:01:01**

ATGGTGTGTCTGAGGCTCCCTGGAGGCTCCTGCATGGCAGTTCTGACAGTGACACTGAT
GGTGCTGAGCTCCCCACTGGCTTTGGCTGGGGACACCAGACCACGTTTCTTGAGTACT
CTACGGGTGAGTGTTATTTCTTCAATGGGACGGAGCGGGTGCGGTTACTGGAGAGACAC
TTCCATAACCAGGAGGAGCTCCTGCGCTTCGACAGCGACGTGGGGGAGTTCCGGGCGGT
GACGGAGCTGGGGCGGCCTGTCGCCGAGTCCTGGAACAGCCAGAAGGACATCCTGGAAG
ACAGGCGCGCCGCGGTGGACACCTATTGCAGACACAACCTACGGGGCTGTGGAGAGCTTC
ACAGTGCAGCGGCGAGTCCATCCTAAGGTGACTGTGTATCCTTCAAAGACCCAGCCCCCT
GCAGCACCACAACCTCCTGGTCTGTTCTGTGAGTGGTTTCTATCCAGGCAGCATTGAAG
TCAGGTGGTTCCGGAATGGCCAGGAAGAGAAGACTGGGGTGGTGTCCACGGGCCTGATC
CACAATGGGAGACTGGACCTTCCAGACCCTGGTGATGCTGGAAACAGTTCCTCGGAGTGG
AGAGGTTTACACCTGCCAAGTGGAGCACCCAAGCGTGACAAGCCCTCTCACAGTGGAAT
GGAGAGCACGGTCTGAATCTGCACAGAGCAAGATGCTGAGTGGAGTCGGGGGCTTTGTG
CTGGGCCTGCTCTTCCTTGGGGCCGGGCTGTTTCATCTACTTCAGGAATCAGAAAGGACA
CTCTGGACTTCAGCCAAGAGGATTCCTGAGCTGA

SEQ_ID_NO:4**Polypeptide sequence of HLA-DRB1*12:01:01:01**

MVCLRLPGGSCMAVLTVTLMVLSSPLALAGDTRPRFLEYSTGECYFFNGTERVRLLEH
FHNQEELLRFDSVDGEFRAVTELGPRVAESWNSQKDILEDRAAVDITYCRHNYGAVESF
TVQRRVHPKVTVYPSKTQPLQHNNLLVCSVSGFYPGSIEVRWFRNGQEEKTGVVSTGLI
HNGDWTFTLVMLETVPRSGEVYTCQVEHPSVTSPLTVEWRARSESAQSKMLSGVGGFV
LGLLFLGAGLFIYFRNQKGHSGLQPRGFLS

FIG. 17 (Cont.)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/037874

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/17; A61P 35/00; C07K 14/725; C12N 5/0783; C12N 5/1; C12N 15/09 (2018.01)

CPC - A61K 35/17; C07K 14/705; C07K 14/7051; C07K 2317/622; C07K 2319/03 (2018.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 800/4 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/075389 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 04 May 2017 (04.05.2017) entire document	1-3, 22-24, 50-53 ----- 68-70
X	US 2008/0226664 A1 (OLD et al) 18 September 2008 (18.09.2008) entire document	89-91
Y	EDES et al. "In Vivo Transduction of T Cells: The Future of Immunotherapy?" Molecular Therapy, 01 May 2016 (01.05.2016), Vol. 24, Supplement 1, Pgs. S201-S202. entire document	68-70
A	WO 2016/033331 A1 (BIOATLA, LLC et al) 03 March 2016 (03.03.2016) entire document	1-3, 22-24, 50-53, 68-70, 89-91
A	WO 2014/186469 A2 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 20 November 2014 (20.11.2014) entire document	1-3, 22-24, 50-53, 68-70, 89-91
A	WO 2013/063361 A1 (REGENERON PHARMACEUTICALS, INC.) 02 May 2013 (02.05.2013) entire document	1-3, 22-24, 50-53, 68-70, 89-91
A	WAN et al. "Probing the Effector and Suppressive Functions of Human T Cell Subsets Using Antigen-Specific Engineered T Cell Receptors," PLoS ONE, 20 February 2013 (20.02.2013), Vol. 8, Iss. 2, Pgs. 1-12. entire document	1-3, 22-24, 50-53, 68-70, 89-91

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 August 2018

Date of mailing of the international search report

17 SEP 2018

Name and mailing address of the ISA/US

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Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2018/037874

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-21, 25-49, 54-67, 71-88, 92-96
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.