vector providing a genetically modified population of immune cells having a higher percentage of cells expressing an exogenous gene product, cell populations comprising a higher percent of exogenous gene product positive cells, and methods of treatment employing populations of cells prepared using the disclosed methods. Further, the described methods provide genetically modified cell populations having a more consistent percentage of cells expressing an exogenous gene product from production run to production run, and/or less expensive and less complex transduction methods. For example, described herein are methods that are particularly suitable for T cell retroviral vector transduction, which can be used for the manufacture of cells useful in allogenic cell therapies employing chimeric antigen receptors (e.g., allogeneic CAR-T cell therapy).

[006] In one example, a method for transducing a population of cells with a retroviral vector where the vector comprises a nucleic acid exogenous to the cells, the method comprising a) selecting the cell population, wherein the selected cell population comprises T lymphocytes, helper T cells, tumor cells, memory T cells, cytotoxic T cells, natural killer T cells, peripheral blood lymphocytes, peripheral blood mononuclear cells, dendritic cells, or natural killer cells or mixtures thereof; and b) culturing the selected cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least the first hour of the transduction culturing step to result in a transduced cell population comprising cells expressing a gene product encoded by the exogenous nucleic acid, is provided.

[006a] In one aspect, the present disclosure refers to a method for transducing a population of cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the cells, the method comprising: a) selecting the cell population, wherein the selected cell population comprises T lymphocytes, helper T cells, tumor cells, memory T cells, cytotoxic T cells, natural killer T cells, peripheral blood lymphocytes, peripheral blood mononuclear cells, dendritic cells, or natural killer cells or mixtures thereof; and b) culturing the selected cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least a first hour of the transduction culturing step to result in a transduced cell population comprising cells expressing a gene product encoded by the exogenous nucleic acid.

[007] In one example, the starting pH of the transduction method is maintained in a starting pH range of 7.0 to 7.9 for at least 1, 2, 4, 6, 8, 10, 12, 16, 18, 20, 22 or 24 hours. In some examples, the starting pH is maintained in the starting pH range for at least about 1, about 2, about 4, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

[008] In some examples, the starting pH is maintained in the starting pH range through the end of the transduction culturing step. In some examples, the transduction culturing step is conducted for at least 1, 2, 4, 6, 8, 12, 14, 16, 18, 20, 22, 24, 36, 48, 72 or 96 hours. In some examples, the pH is controlled passively. In some examples, the pH is controlled actively with a bioreactor.

[009] In some examples, the transduction method of the instant disclosure comprises culturing the selected cell population at a MOI of about .25, about .5, about 1, about 5,

about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250. In some examples the MOI is a ratio of functional viral particles to total number of target cells in a transduction procedure. In some examples, the titer of the functional viral particles to be added to a transduction procedure is determined by qPCR.

[010] In some examples of the instant disclosure, the exogenous nucleic acid encodes a chimeric antigen receptor (CAR). In some examples the exogenous nucleic acid encodes an epitope specific for a monoclonal antibody, a suicide polypeptide, an inducible "on" or "accelerator" switch or a control switch, e.g., a dimerization domain.

[011] In some examples, the selected cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag. In some examples, the transduction culturing step comprises culturing the selected cell population and the retroviral vector in a volume of about .75 liters to a volume of about 250 liters of cell culture medium. In some examples, the transduction culturing step comprises culturing the selected cell population and the retroviral vector in a volume of about .5 liters to a volume of about 10 liters of cell culture medium.

[012] In some examples, the retroviral vector used in the methods of instant disclosure is a lentiviral vector.

[013] In some examples, at least 35% to 95%, 40% to 95%, 50% to 95%, 60% to 95%, 70% to 95%, of the transduced cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step. In some examples, at least 35% to 95%, 40% to 95%, 50% to 95%, 60% to 95%, 70% to 95%, of the transduced cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

[014] In some examples, the cell culture media optionally comprises a co-localization agent during the transduction culturing step, such as fibronectin, fibronectin derivatives, polybrene or RetroNectin reagent, during the transduction culturing step.

[015] In some examples, the cell culture media does not comprise a co-localization agent, such as fibronectin or a fibronectin derivative, e.g., RetroNectin reagent, during the transduction culturing step.

[016] In some examples, the cell culture media does not comprise a co-localization

agent during the transduction culturing step.

[017] In some examples, a polycation, such as polybrene, protamine sulfate or DEAE-dextran, is not added to the cell culture media prior to or during transduction.

[018] In some examples, the selected cell population is an allogeneic cell population. In some examples, the selected cell population is an autologous cell population.

[019] In one example, a method for transducing a first and a second cell population, wherein the first and second cell population are transduced by the same method of the instant disclosure and whereby the percent of the transduced cell population expressing the exogenous nucleic acid gene product in the first and second transduced cell population does not vary more than 2% to 5%, 5% to 10%, 10% to 20%, or 20% to 30%, is provided.

[019a] In another aspect, the present disclosure refers to a method for transducing a first and a second cell population, wherein the first and second cell population are transduced by the same method as disclosed herein, whereby the percent of the transduced cell population expressing the exogenous nucleic acid gene product in the first and second transduced cell populations does not vary more than about 2% to about 5%, about 5% to about 10%, about 10% to about 20%, or about 20% to about 30%.

[020] In some examples, the methods of the instant disclosure provide a transduced cell population wherein a cell of the transduced cell population comprises a vector copy number which is reduced compared to a cell transduced by the same method of the instant disclosure wherein the starting pH of the transduction culturing step of the same method is less than 7.0.

[021] In an example of the instant disclosure, a method for transducing a population of cytotoxic T cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the cytotoxic T cells, the method comprising culturing the cytotoxic T cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least the first hour of the transduction culturing step to result in a transduced cytotoxic T cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent, is provided.

[021a] In another aspect, the present disclosure refers to a method for transducing a population of cytotoxic T cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the cytotoxic T cells, the method comprising culturing the cytotoxic T cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least a first hour of the transduction culturing step to result in a transduced cytotoxic T cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent.

[022] In some examples of this example of the instant disclosure, the starting pH is maintained in a starting pH range of 7.0 to 7.9 for at least 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24 hours. In some examples, the starting pH of the cytotoxic T cell culture media is maintained in the starting pH range for at least about 1, about 2, about 4, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

[023] In some examples of this example, the starting pH of the cytotoxic T cell culture media is maintained in the starting pH range through the end of the transduction culturing step. In some examples, the transduction culturing step is conducted for at least 1, 2, 4, 6, 8, 12, 14, 16, 18, 20, 22, 24, 36, 48, 72 or 96 hours.

[024] In some examples of this example of the instant disclosure the pH is controlled passively. In some examples, the pH is controlled actively.

[025] In some examples, the cytotoxic T cell culture media does not comprise a co-localization agent, such as fibronectin or a fibronectin derivative, e.g., RetroNectin® during the transduction culturing step.

[026] In some examples, a polycation, such as polybrene, protamine sulfate or DEAE-dextran, is not added to the cytotoxic T cell culture media prior to or during transduction.

[027] In some examples of this example of the instant disclosure, the cytotoxic T cell population is cultured at a MOI of about .25, about .5, about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250. In some examples the MOI is a ratio of functional viral particles to total number of cytotoxic T cells in a transduction procedure.

[028] In some examples of this example of the instant disclosure, the exogenous nucleic acid encodes a chimeric antigen receptor. In some examples the exogenous nucleic acid encodes an epitope specific for a monoclonal antibody, a suicide polypeptide, an inducible "on" or "accelerator" switch or a control switch, e.g., a dimerization domain.

[029] In some examples of this example of the instant disclosure, the cytotoxic T cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag. In some examples, the transduction culturing step comprises culturing the cytotoxic T cell population and the retroviral vector in a volume of about .75 liters to a volume of about 250 liters of cell culture media.

[030] In some examples, the retroviral vector for use in the methods of instant disclosure is a lentiviral vector.

[031] In some examples of this example of the instant disclosure, at least 40% to 95%, 50% to 95%, 60% to 95%, 70% to 95%, of the transduced cytotoxic T cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step. In some examples, at least 40% to 95%, 50% to 95%, 60% to 95%, 70% to 95%, of the transduced cytotoxic T cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

[032] In some examples, the cytotoxic T cell population to be transduced is an

allogeneic cytotoxic T cell population. In some examples, the cytotoxic T cell population to be transduced is an autologous cytotoxic T cell population.

[033] In an example of the instant disclosure, a method for transducing a first and a second cytotoxic T cell population, wherein the first and second cytotoxic T cell population are transduced by the same method of the instant disclosure whereby the percent of the transduced cytotoxic T cell population expressing the exogenous nucleic acid gene product in the first and second transduced cytotoxic T cell population does not vary more than 2% to 5%, 5% to 10%, 10% to 20%, or 20% to 30%, is provided.

[033a] In another aspect, the present disclosure refers to a method for transducing a first and a second cytotoxic T cell population, wherein the first and second cytotoxic T cell population are transduced by the method as disclosed herein, whereby the percent of the transduced cytotoxic T cell population expressing the exogenous nucleic acid gene product in the first and second transduced cytotoxic T cell populations does not vary more than about 2% to about 5%, about 5% to about 10%, about 10% to about 20%, or about 20% to about 30%.

[034] In an example of the instant disclosure, a method for transducing a population of peripheral blood mononuclear cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the peripheral blood mononuclear cells, the method comprising culturing the peripheral blood mononuclear cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least the first hour of the transduction culturing step to result in a transduced peripheral blood mononuclear cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent, is provided.

[034a] In another aspect, the present disclosure refers to a method for transducing a population of peripheral blood mononuclear cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the peripheral blood mononuclear cells, the method comprising culturing the peripheral blood mononuclear cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least a first hour of the transduction culturing step to result in a transduced peripheral blood mononuclear cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent.

[035] In some examples of this example of the instant disclosure, the starting pH is maintained in a starting pH range of 7.0 to 7.9 for at least 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24 hours. In some examples, the starting pH is maintained in the starting pH range for at least about 1, about 2, about 4, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

[036] In some examples of this example, the starting pH is maintained in the starting pH range through the end of the transduction culturing step. In some examples, the transduction culturing step is conducted for at least 1, 2, 4, 6, 8, 12, 14, 16, 18, 20, 22, 24, 36, 48, 72 or 96 hours.

[037] In some examples of this example of the instant disclosure the pH is controlled passively. In some examples, the pH is controlled actively.

[038] In some examples, the peripheral blood mononuclear cell culture media does not comprise a co-localization agent, such as fibronectin or a fibronectin derivative, e.g., RetroNectin® during the transduction culturing step.

[039] In some examples, a polycation, such as polybrene, protamine sulfate or DEAE-

dextran, is not added to the peripheral blood mononuclear cell culture media prior to or during transduction.

[040] In some examples of this example of the instant disclosure, the peripheral blood mononuclear cell population is cultured at a MOI of about .25, about .5, about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250. In some examples the MOI is a ratio of functional viral particles to total number of peripheral blood mononuclear cells in a transduction procedure.

[041] In some examples of this example of the instant disclosure, the exogenous nucleic acid encodes a chimeric antigen receptor. In some examples the exogenous nucleic acid encodes an epitope specific for a monoclonal antibody, a suicide polypeptide, an inducible "on" or "accelerator" switch or a control switch, e.g., a dimerization domain.

[042] In some examples of this example of the instant disclosure, the peripheral blood mononuclear cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag. In some examples, the transduction culturing step comprises culturing the peripheral blood mononuclear cell population and the retroviral vector in a volume of about .75 liters to a volume of about 250 liters of cell culture media.

[043] In some examples, the retroviral vector for use in the methods of this example of the instant disclosure is a lentiviral vector.

[044] In some examples of this example of the instant disclosure, at least 40% to 95%, 50% to 95%, 60% to 95%, 70% to 95%, of the transduced peripheral blood mononuclear cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step. In some examples, at least 40% to 95%, 50% to 95%, 60% to 95%, 70% to 95%, of the transduced peripheral blood mononuclear cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

[045] In some examples, the peripheral blood mononuclear cell population to be transduced is an allogeneic peripheral blood mononuclear cell population. In some examples, the peripheral blood mononuclear cell population to be transduced is an autologous peripheral blood mononuclear cell population.

[046] In an example of the instant disclosure, a method for transducing a first and a second

peripheral blood mononuclear cell population, wherein the first and second peripheral blood mononuclear cell population are transduced by the same method of the instant disclosure whereby the percent of the transduced peripheral blood mononuclear cell population expressing the exogenous nucleic acid gene product in the first and second transduced peripheral blood mononuclear cell populations does not vary more than 2% to 5%, 5% to 10%, 10% to 20%, or 20% to 30%, is provided.

[046a] In another aspect, the present disclosure refers to a method for transducing a first and a second peripheral blood mononuclear cell population, wherein the first and second peripheral blood mononuclear cell populations are transduced by the same method as disclosed herein, whereby the percent of the transduced peripheral blood mononuclear cell population expressing the exogenous nucleic acid gene product in the first and second transduced peripheral blood mononuclear cell population does not vary more than about 2% to about 5%, about 5% to about 10%, about 10% to about 20%, or about 20% to about 30%.

[047] In an example of the instant disclosure, a method for transducing a population of T cells derived from induced pluripotent stem cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the derived T cells, the method comprising culturing the derived T cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least the first hour of the transduction culturing step to result in a transduced derived T cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent, is provided.

[047a] In another aspect, the present disclosure refers to a method for transducing a population of T cells derived from induced pluripotent stem cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the derived T cells, the method comprising culturing the derived T cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least t a first hour of the transduction culturing step to result in a transduced derived T cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent.

[048] In some examples of this example of instant disclosure, the starting pH is maintained in a starting pH range of 7.0 to 7.9 for at least 1, 2, 4, 6, 8, 10, 12, 16, 18, 20, 22 or 24 hours. In some examples, the starting pH of the culture media for the T cell population derived from induced pluripotent stem cells is maintained in the starting pH range for at least about 1, about 2, about 4, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

[049] In some examples of this example, the starting pH of the derived T-cell culture media is maintained in the starting pH range through the end of the transduction culturing step. In some examples, the transduction culturing step is conducted for at least 1, 2, 4, 6, 8, 12, 14, 16, 18, 20, 22, 24, 36, 48, 72 or 96 hours.

[050] In some examples of this example of the instant disclosure the pH of the derived T-cell culture media is controlled passively. In some examples, the pH is controlled actively.

[051] In some examples of this example of the invention, the derived T-cell culture media does not comprise a co-localization agent, such as fibronectin or a fibronectin derivative, e.g., RetroNectin® during the transduction culturing step.

[052] In some examples, a polycation, such as polybrene, protamine sulfate or DEAE-

dextran, is not added to the derived T-cell culture media prior to or during transduction.

[053] In some examples of this example of the instant disclosure, the derived T cell population is cultured at a MOI of about .25, about .5, about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250. In some examples the MOI is a ratio of functional viral particles to the total number of T cells derived from induced pluripotent stem cells in a transduction procedure.

[054] In some examples of this example of the instant disclosure, the exogenous nucleic acid encodes a chimeric antigen receptor. In some examples the exogenous nucleic acid encodes an epitope specific for a monoclonal antibody, a suicide polypeptide, an inducible "on" or "accelerator" switch or a control switch, e.g., a dimerization domain.

[055] In some examples of this example of the instant disclosure, the derived T cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag. In some examples, the transduction culturing step comprises culturing the derived T cell population and the retroviral vector in a volume of about 0.5 liters to a volume of about 10 liters of cell culture media.

[056] In some examples, the retroviral vector for use in the methods of instant disclosure is a lentiviral vector.

[057] In some examples of this example of the instant disclosure, 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, or about 70% to about 95%, of the transduced derived T cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step. In some examples, about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, or about 70% to about 95%, of the transduced derived T cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

[058] In some examples, the derived T cell population to be transduced is an allogeneic derived T cell population. In some examples, the derived T cell population to be transduced is an autologous derived T cell population.

[059] In an example of the instant disclosure, a method for transducing a first and a second derived T cell population, wherein the first and second derived T cell population are

transduced by the same method of the instant disclosure whereby the percent of the transduced derived T cell population expressing the exogenous nucleic acid gene product in the first and second transduced derived T cell population does not vary more than 2% to 5%, 5% to 10%, 10% to 20%, or 20% to 30%, is provided.

[059a] In another aspect, the present disclosure refers to a method for transducing a first and a second derived T cell population, wherein the first and second derived T cell population are transduced by the same method as disclosed herein, whereby the percent of the transduced derived T cell population expressing the exogenous nucleic acid gene product in the first and second transduced derived T cell population does not vary more than 2% to 5%, 5% to 10%, 10% to 20%, or 20% to 30%.

[060] In some examples of the present invention, a genetically modified cell produced by the methods of instant disclosure is provided. In another example of the present invention, a population of genetically modified cells produced by the methods of instant disclosure is provided. In another example, a therapeutic composition comprising a cell or cell population produced by the methods of the invention is provided. In another example, a method of treatment comprising administering a therapeutic effective amount of a cell, cell population or therapeutic composition produced by the methods of the instant invention to a subject in need thereof is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

[061] Fig. 1 shows exemplary protocols for isolating PBMCs, activating, transducing, transfecting, expanding, and harvesting T cells from the isolated PBMCs.

[062] Fig 2A and 2B show that the averaged percentage of viable CAR+ T cells, on Day 11 of the study, when the cells are transduced at pH of 7.1 (Fig. 2A) is 81.28587 or 23.0387 when transduced at pH 6.6 (Fig. 2B), as determined by ANOVA analysis.

[063] Fig. 2C shows the p-values for the main effects and 2-way interactions of pH, % lentivirus vector (v/v) (indicated as LVV), RetroNectin® reagent concentration (μg/mL) (indicated as RN), and vessel type on the percentage of viable CAR+ T cells.

[064] Fig. 3 shows the effect of 1, 2-, 4-, 6- and 8-hour transduction times (horizontal axis) carried out a pH of 7.2 ± 0.1, on the percentage of CAR+ T cells (vertical axis) on Day 7 and Day 14 of the study.

DETAILED DESCRIPTION

[065] Provided herein, are improved methods for transducing immune cells, particularly T-cells, that increase transduction efficiency and/or improve the consistency of exogenous nucleic acid expression levels between production runs while reducing processing costs and complexity.

[066] Illustrative methods provided herein, which are typically ex vivo methods, include

WHAT IS CLAIMED IS

1. A method for transducing a population of cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the cells, the method comprising:

a) selecting the cell population, wherein the selected cell population comprises T lymphocytes, helper T cells, tumor cells, memory T cells, cytotoxic T cells, natural killer T cells, peripheral blood lymphocytes, peripheral blood mononuclear cells, dendritic cells, or natural killer cells or mixtures thereof; and

b) culturing the selected cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least a first hour of the transduction culturing step to result in a transduced cell population comprising cells expressing a gene product encoded by the exogenous nucleic acid.

2. The method according to claim 1, wherein the starting pH is maintained in the starting pH range for at least about 1, about 2, about 4, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

3. The method according to claim 1, wherein the starting pH is maintained above about 7.0 through the end of the transduction culturing step.

4. The method according to claim 3, wherein the transduction culturing step is conducted for at least about 1, about 2, about 4, about 6, about 8, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours.

5. The method according to any one of the preceding claims, wherein the cell culture media does not comprise a co-localization agent.

6. The method according to any one of the preceding claims, wherein the cell culture media does not comprise fibronectin or a fibronectin derivative.

7. The method according to any one of the preceding claims, wherein the selected cell

population is cultured at a MOI of about .25, about .5, about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250.

8. The method according to any one of the preceding claims, wherein the exogenous nucleic acid encodes a chimeric antigen receptor.

9. The method according to any one of the preceding claims, wherein the selected cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag.

10. The method according to any one of the preceding claims, wherein the retroviral vector is a lentiviral vector.

11. The method according to any one of the preceding claims, wherein the transduction culturing step comprises culturing the selected cell population and the retroviral vector in a volume of about 0.5 liters to a volume of about 10 liters of cell culture media.

12. The method according to any one of the preceding claims, wherein, at least about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, or about 70% to about 95%, of the transduced cell population expresses the exogenous nucleic acid gene product about 3 to about 18 days after initiation of the transduction culturing step.

13. The method according to any one of the preceding claims, wherein, at least about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, or about 70% to about 95%, of the transduced cell population expresses the exogenous nucleic acid gene product about 7 to about 18 days after initiation of the transduction culturing step.

14. The method according to any one of the preceding claims, wherein the starting pH range is maintained passively.

15. The method according to any one of claims 1 to 13, wherein the starting pH range is maintained actively.

16. The method according to any of the preceding claims, wherein the selected cell population is an allogeneic cell population.

17. The method according to any one of claims 1-15, wherein the selected cell population is an autologous cell population.

18. A method for transducing a first and a second cell population, wherein the first and second cell population are transduced by the same method of any one of the preceding claims, whereby the percent of the transduced cell population expressing the exogenous nucleic acid gene product in the first and second transduced cell populations does not vary more than about 2% to about 5%, about 5% to about 10%, about 10% to about 20%, or about 20% to about 30%.

19. The method according to any one of the preceding claims, wherein a polycation is not added to the cell culture media.

20. The method according to any one of the preceding claims, wherein polybrene, protamine sulfate or DEAE-dextran is not added to the culture media.

21. A method for transducing a population of cytotoxic T cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the cytotoxic T cells, the method comprising culturing the cytotoxic T cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least a first hour of the transduction culturing step to result in a transduced cytotoxic T cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent.

22. The method according to claim 21, wherein the starting pH is maintained in a starting pH range of 7.0 to 7.9 for at least about 1, about 2, about 6, about 8, about 10, about 12,

about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

23. The method according to claim 21, wherein the starting pH is maintained in the starting pH range through the end of the transduction culturing step.

24. The method according to claim 23, wherein the transduction culturing step is conducted for at least about 1, about 2, about 4, about 6, about 8, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours.

25. The method according to anyone of the preceding claims 21 to 24, wherein the co-localization agent is fibronectin or a fibronectin derivative.

26. The method according to any one of the preceding claims 21 to 25 , wherein the cytotoxic T cell population is cultured at a MOI of about .25, about .5, about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250.

27. The method according to any one of the preceding claims 21 to 26, wherein the exogenous nucleic acid encodes a chimeric antigen receptor.

28. The method according to any one of the preceding claims 21 to 27, wherein the cytotoxic T cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag.

29. The method according to any one of the preceding claims 21 to 28, wherein the retroviral vector is a lentiviral vector.

30. The method according to any one of the preceding claims 21 to 29, wherein the transduction culturing step comprises culturing the cytotoxic T cell population and the retroviral vector in a volume of about 0.5 liters to a volume of about 10 liters of cell culture

media.

31. The method according to any one of the preceding claims 21 to 30, wherein, 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, or about 70% to about 95%, of the transduced cytotoxic T cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step.

32. The method according to any one of the preceding claims 21 to 31, wherein, 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, or about 70% to about 95%, of the transduced cytotoxic T cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

33. The method according to any one of the preceding claims 21 to 32, wherein the starting pH range is maintained passively.

34. The method according to any one of claims 21 to 32, wherein the starting pH range is maintained actively.

35. The method according to any of the preceding claims 21 to 34, wherein the cytotoxic T cell population is an allogeneic cytotoxic T cell population.

36. The method according to any one of claims 21 to 34, wherein the cytotoxic T cell population is an autologous cytotoxic T cell population.

37. A method for transducing a first and a second cytotoxic T cell population, wherein the first and second cytotoxic T cell population are transduced by the method of any one of the preceding claims 21 to 36, whereby the percent of the transduced cytotoxic T cell population expressing the exogenous nucleic acid gene product in the first and second transduced cytotoxic T cell populations does not vary more than about 2% to about 5%, about 5% to about 10%, about 10% to about 20%, or about 20% to about 30%.

38. The method according to any one of the preceding claims 21 to 37, wherein a polycation is not added to the cell culture media.

39. The method according to any one of the preceding claims 21 to 38, wherein polybrene, protamine sulfate or DEAE-dextran is not added to the culture media.

40. A method for transducing a population of peripheral blood mononuclear cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the peripheral blood mononuclear cells, the method comprising culturing the peripheral blood mononuclear cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least a first hour of the transduction culturing step to result in a transduced peripheral blood mononuclear cell population comprising cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent.

41. The method according to claim 40, wherein the starting pH is maintained in a starting pH range of 7.0 to 7.9 for at least about 1, about 2, about 4, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24 hours, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

42. The method according to claim 40, wherein the starting pH is maintained in the starting pH range through the end of the transduction culturing step.

43. The method according to claim 42, wherein the transduction culturing step is conducted for at least about 1, about 2, about 4, about 6, about 8, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours.

44. The method according to anyone of the preceding claims 40 to 43, wherein the co-localization agent is fibronectin or a fibronectin derivative.

45. The method according to any one of the preceding claims 40 to 44, wherein the peripheral blood mononuclear cell population is cultured at a MOI of about .25, about .5,

about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250.

46. The method according to any one of the preceding claims 40 to 45, wherein the exogenous nucleic acid encodes a chimeric antigen receptor.

47. The method according to any one of the preceding claims 40 to 46, wherein the peripheral blood mononuclear cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag.

48. The method according to any one of the preceding claims 40 to 47, wherein the retroviral vector is a lentiviral vector.

49. The method according to any one of the preceding claims 40 to 48, wherein the transduction culturing step comprises culturing the peripheral blood mononuclear cell population and the retroviral vector in a volume of about 0.5 liters to a volume of about 10 liters of cell culture media.

50. The method according to any one of the preceding claims 40 to 49, wherein, at least about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, about 70% to about 95%, of the transduced peripheral blood mononuclear cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step.

51. The method according to any one of the preceding claims 40 to 50, wherein, at least about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, about 70% to about 95%, of the transduced peripheral blood mononuclear cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

52. The method according to any one of the preceding claims 40 to 51, wherein the starting pH range is maintained passively.

53. The method according to any one of claims 40 to 52, wherein the starting pH range is maintained actively.

54. The method according to any of the preceding claims 40 to 53, wherein the peripheral blood mononuclear cell population is an allogeneic peripheral blood mononuclear cell population.

55. The method according to any one of claims 40 to 54, wherein the peripheral blood mononuclear cell population is an autologous peripheral blood mononuclear cell population.

56. A method for transducing a first and a second peripheral blood mononuclear cell population, wherein the first and second peripheral blood mononuclear cell populations are transduced by the same method of any one of the preceding claims 40 to 55, whereby the percent of the transduced peripheral blood mononuclear cell population expressing the exogenous nucleic acid gene product in the first and second transduced peripheral blood mononuclear cell population does not vary more than about 2% to about 5%, about 5% to about 10%, about 10% to about 20%, or about 20% to about 30%.

57. The method according to any one of the preceding claims 40 to 56, wherein a polycation is not added to the cell culture media.

58. The method according to any one of the preceding claims 40 to 57, wherein polybrene, protamine sulfate or DEAE-dextran is not added to the culture media.

59. A method for transducing a population of T cells derived from induced pluripotent stem cells with a retroviral vector, the vector comprising a nucleic acid exogenous to the derived T cells, the method comprising culturing the derived T cell population with the retroviral vector, in a cell culture media at starting pH in a starting pH range of 7.0 to 7.9 and maintaining the starting pH in the starting pH range for at least t a first hour of the transduction culturing step to result in a transduced derived T cell population comprising

cells expressing the gene product encoded by the exogenous nucleic acid and wherein the cell culture media does not comprise a co-localization agent.

60. The method according to claim 59, wherein the starting pH is maintained in a starting pH range of 7.0 to 7.9 for at least about 1, about 2, about 6, about 8, about 10, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours to no more than about 168 hours.

61. The method according to claim 59, wherein the starting pH is maintained in the starting pH range through the end of the transduction culturing step.

62. The method according to claim 61, wherein the transduction culturing step is conducted for at least about 1, about 2, about 4, about 6, about 8, about 12, about 14, about 16, about 18, about 20, about 22, about 24, about 36, about 48, about 72 or about 96 hours.

63. The method according to anyone of the preceding claims 59 to 62, wherein the co-localization agent is fibronectin or a fibronectin derivative.

64. The method according to any one of the preceding claims 59 to 63, wherein the derived T cell population is cultured at a MOI of about .25, about .5, about 1, about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about, or about 50 to about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 125, about 150, about 175, about 200, about 225, or about 250. .

65. The method according to any one of the preceding claims 59 to 64, wherein the exogenous nucleic acid encodes a chimeric antigen receptor.

66. The method according to any one of the preceding claims 59 to 65, wherein the derived T cell population is cultured in a vessel, wherein the vessel is a cell culture plate, cell culture deep well plate, a cell stack, a controlled bioreactor, a shake flask or a gas permeable bag.

67. The method according to any one of the preceding claims 59 to 66, wherein the

retroviral vector is a lentiviral vector.

68. The method according to any one of the preceding claims 59 to 67, wherein the transduction culturing step comprises culturing the derived T cell population and the retroviral vector in a volume of about 0.5 liters to a volume of about 10 liters of cell culture media.

69. The method according to any one of the preceding claims 59 to 68, wherein, at least about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, about 70% to about 95%, of the transduced derived T cell population expresses the exogenous nucleic acid gene product 3 to 18 days after initiation of the transduction culturing step.

70. The method according to any one of the preceding claims 59 to 69, wherein, at least about 35% to about 95%, about 40% to about 95%, about 50% to about 95%, about 60% to about 95%, about 70% to about 95%, of the transduced derived T cell population expresses the exogenous nucleic acid gene product 7 to 18 days after initiation of the transduction culturing step.

71. The method according to any one of the preceding claims 59 to 70, wherein the starting pH range is maintained passively.

72. The method according to any one of claims 59 to 70, wherein the starting pH range is maintained actively.

73. The method according to any of the preceding claims 59 to 72, wherein the derived T cell population is an allogeneic derived T cell population.

74. The method according to any one of claims 59 to 72, wherein the derived T cell population is an autologous derived T cell population.

75. A method for transducing a first and a second derived T cell population, wherein the first and second derived T cell population are transduced by the same method of any one of

the preceding claims 59 to 74, whereby the percent of the transduced derived T cell population expressing the exogenous nucleic acid gene product in the first and second transduced derived T cell population does not vary more than 2% to 5%, 5% to 10%, 10% to 20%, or 20% to 30%.

76. The method according to any one of the preceding claims 59 to 75, wherein a polycation is not added to the cell culture media.

77. The method according to any one of the preceding claims 59 to 76, wherein polybrene, protamine sulfate or DEAE-dextran is not added to the culture media.