domain is linked to an intracellular signaling domain that activates the T cell upon antigen binding. Such CAR T cells can provide a powerful tool to combat pathogens and cancer cells because upon binding to the target antigen in vivo, the CAR T cells undergo further expansion and activation to provide a type of "living drug" that can have a direct cytotoxic action against the target as well as influence the endogenous immune functionality through production of cytokines.

While adoptive T cell therapy is theoretically versatile and can be specifically applied to address a variety of conditions, such as cancers, infectious diseases, and autoimmune diseases, there is a growing recognition that achievement of durable clinical responses to the treatment depends on qualities of the transferred T cells that result in proliferation, persistence, and resistance to exhaustion in the tumor microenvironment in the subject after administration. The factors that determine robust T cell expansion *ex vivo* and *in vivo*, subsequent persistence *in vivo*, the durability of the T cell response *in vivo*, and T cell toxicities *in vivo* have been challenging to define both in the starting population of T cells that are propagated *ex vivo* and in the final product that is infused to the patient. This challenge has been due in part to variations in the phenotypic composition of T cells isolated from the source, in particular for patients with malignancies or hyperproliferative disorders of the immune system, such as lymphoma and myeloma.

Accordingly, despite the advances in the art of adoptive T cell therapy, a need remains for methods and compositions that consistently provide robust T cells with enhanced therapeutic efficacy, activity, and persistence in a subject after administration. The present disclosure addresses this and related needs.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

In one aspect, the disclosure provides a method of culturing naïve T (T_N) cells *in vitro*, comprising exposing a population of naïve T(T_N) cells to a medium comprising Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell, wherein the population of T_N cells comprises at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% of T_N cells.

In another aspect, the disclosure provides an *in vitro* method of generating a T_N cell expressing a heterologous immune receptor, comprising: performing the method recited in the disclosure; and during the exposing step, transducing the T_N cell with a heterologous nucleic acid molecule comprising a sequence that encodes an immune receptor.

In another aspect, the disclosure provides a method of reducing or preventing exhaustion of a T_N cell expressing a chimeric antigen receptor or of a population of T_N cells

expressing a chimeric antigen receptor, the method comprising exposing the T_N cell or the population of T_N cells to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell.

In another aspect, the disclosure provides a method of generating a T_N cell or a population of T_N cells expressing a chimeric antigen receptor, the method comprising modifying the T_N cell or the population of T_N cells to express a chimeric antigen receptor to a medium comprising a Notch receptor agonist, wherein the Notch receptor agonist reduces or prevents exhaustion of the T_N cell.

In one ~~aspect~~example, the disclosure provides a method of culturing a T cell *in vitro*. The method comprises exposing a T cell to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell.

In some ~~embodiments~~examples, the T cell is not cultured in the presence of an antigen-presenting cell (APC). In some ~~embodiment~~examples, the Notch receptor agonist is not

expressed on a cell in the medium. In some ~~embodiment~~examples, the T cell is not cultured in the presence of an antigen-presenting cell (APC) expressing a Notch receptor agonist. For example, in some ~~embodiment~~examples the Notch receptor agonist is not expressed on an APC in the medium. In some ~~embodiment~~examples, the T cell is not cultured in the presence of an antigen-presenting cell (APC) expressing Delta like ligand 4 (DLL4).

In some ~~embodiment~~examples, the T cell is a naïve T cell (T_N), a memory stem T cell (T_{SCM}), or a central memory T cell (T_{CM}). In one ~~embodiment~~example, the T cell is a naïve T cell (T_N). In some ~~embodiment~~examples, the T cell is an effector memory T cell (T_{EM}). In some ~~embodiment~~examples, the T cell is further characterized as CD62L+. In some ~~embodiment~~examples, the T cell is further characterized as CD45RA+. In some ~~embodiment~~examples, the T cell is further characterized as CD45RO. In some ~~embodiment~~examples, the T cell is further characterized as CD95. In some ~~embodiment~~examples, the T cell is further characterized as CCR7+. In some ~~embodiment~~examples, the T cell is further characterized as CD62L+, CD45RA+, and CD45RO.

In another ~~aspect~~example, the disclosure provides a method of culturing T cells *in vitro*, comprising exposing a population of cells to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cells.

In some ~~embodiment~~examples, the T cell population is not cultured in the presence of an antigen-presenting cell (APC). In some ~~embodiment~~examples, the Notch receptor agonist is not expressed on a cell in the medium. In some ~~embodiment~~examples, the T cell population is not cultured in the presence of an antigen-presenting cell (APC) expressing a Notch receptor agonist. For example, in some ~~embodiment~~examples, the Notch receptor agonist is not expressed on an APC in the medium. In some ~~embodiment~~examples, the T cell population is not cultured in the presence of an antigen-presenting cell (APC) expressing Delta like ligand 4 (DLL4).

In one ~~embodiment~~example, the method is a method of culturing naïve T (T_N) cells *in vitro*, that comprises exposing a population of naïve T (T_N) cells to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell. In some ~~embodiment~~examples, the population of T_N cells comprises at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% of T_N cells. In some ~~embodiment~~examples, the population comprises about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 80% to about 90%, about 40% to about 80%, about 50% to about 80%, about 60% to about 80%, about 70% to about 80%, about

40% to about 70%, about 50% to about 70%, about 60% to about 70%, about 40% to about 60%, about 50% to about 60%, or about 40% to about 50% of T_N cells. In some ~~embodiment~~examples, the T_N cell is further characterized as CD62L+, CD45RA+, CD45RO-, CD95-, and/or CCR7+. In some ~~embodiment~~examples, the exposing lasts for a period ("the exposure time") of at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least about six days, at least about a week, at least about eight days, at least about nine days, at least about ten days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, at least about 30 days, or at least about a month. In some ~~embodiment~~examples, the exposure time is between one day to 15 days or between two days to 10 days.

In some ~~embodiment~~examples, the percentage of the T_N cells in the population does not change after the exposing. In some ~~embodiment~~examples, the percentage of the T_N cells in the population changes, after the exposing, by less than about 1%, less than about 2%, less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, or less than about 50%. In some ~~embodiment~~examples, the percentage of the T_N cells in the population is: (i) at least about 40% before the exposing and at least about 40% after the exposing; (ii) at least about 50% before the exposing and at least about 40% after the exposing; (iii) at least about 50% before the exposing and at least about 50% after the exposing; (iv) at least about 50% before the exposing and at least about 60% after the exposing; (v) at least about 60% before the exposing and at least about 50% after the exposing; (vi) at least about 60% before the exposing and at least about 60% after the exposing; (vii) at least about 60% before the exposing and at least about 70% after the exposing; (viii) at least about 70% before the exposing and at least about 60% after the exposing; (ix) at least about 70% before the exposing and at least about 70% after the exposing; (x) at least about 70% before the exposing and at least about 80% after the exposing; (xi) at least about 80% before the exposing and at least about 70% after the exposing; (xii) at least about 80% before the exposing and at least about 80% after the exposing; (xiii) at least about 80% before the exposing and at least about 90% after the

exposing; (xiv) at least about 90% before the exposing and at least about 80% after the exposing; (xv) at least about 90% before the exposing and at least about 90% after the exposing; or (xvi) at least about 90% before the exposing and about 100% after the exposing. In some ~~embodiment~~examples, the T_N cell, the population of T_N cells, or one or more progeny cells thereof, maintains at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, or at least 7.0 fold less-differentiated state *in vivo* compared to the T_N cell that did not receive the Notch receptor agonist.

In some ~~embodiment~~examples of the above ~~aspect~~examples, the Notch receptor agonist comprises a domain of a mammalian Notch receptor ligand that binds to a mammalian Notch1, Notch2, Notch3, or Notch4 receptor. In some ~~embodiment~~examples, the Notch receptor agonist is or comprises a Delta protein, a Jagged protein, an anti-Notch antibody, or a fragment or derivative thereof, that binds to a mammalian Notch receptor, or any combination thereof. In some ~~embodiment~~examples, the Notch receptor agonist, upon binding to a Notch receptor, is capable of exposing an S2 cleavage site in the negative regulatory region (NRR) of the Notch receptor. In some ~~embodiment~~examples, the Notch receptor agonist, upon binding to a Notch receptor, is capable of exposing an S2 cleavage site in the negative regulatory region (NRR) of the Notch receptor.

In some ~~embodiment~~examples, the Notch receptor agonist comprises an extracellular domain of a Delta protein or a Jagged protein. In some ~~embodiment~~examples, the Delta protein is or comprises Delta Like Ligand 1 (DLL1), or an extracellular Notch-binding domain thereof. In some ~~embodiment~~examples, the Delta protein is or comprises Delta Like Ligand 3 (DLL3, or an extracellular Notch-binding domain thereof. In some ~~embodiment~~examples, the Delta protein is or comprises Delta Like Ligand 4 (DLL4), or an extracellular Notch-binding domain thereof. In some ~~embodiment~~examples, the Jagged protein is or comprises Jagged1, or an extracellular Notch-binding domain thereof. In some ~~embodiment~~examples, the Jagged protein is or comprises Jagged2, or an extracellular Notch-binding domain thereof. In some ~~embodiment~~examples, the Notch agonist comprises Dlk1, Dlk2, DNER, EGFL 7, or F3/contactin or a Notch-binding derivative thereof. In some ~~embodiment~~examples, the Notch receptor agonist is an anti-Notch antibody, or derivative thereof, that binds to an epitope

in the Notch extracellular domain (NECD) that is not in the negative regulatory region (NRR) of the Notch receptor. In some ~~embodiment~~examples, the anti-Notch antibody (or antigen binding fragment or derivative thereof) binds to an epitope in the Notch extracellular domain (NECD) that is not in the negative regulatory region (NRR) of Notch1, Notch2, Notch3, and/or Notch4 receptor. In some ~~embodiment~~examples, the Notch receptor agonist is immobilized at a concentration of about 0.01 μg/ml to about 100 μg/ml. In some ~~embodiment~~examples, a plurality of T cells is exposed to the medium at a concentration sufficient to contact substantially all of the immobilized Notch receptor agonist.

In some ~~embodiment~~examples, the medium further comprises one or more cytokines, or a biologically active fragment thereof, that modulate T cell differentiation. In some ~~embodiment~~examples, the one or more cytokines include IL-1, IL-1b, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-21, IL-23, IL-27, IFN-γ, TNF-α, TGFβ, or any combination thereof, in an effective concentration. In some ~~embodiment~~examples, the exposure time of the T cell to the medium is between about 1 and about 15 days. In some ~~embodiment~~examples, the exposure time is between about 2 and about 10 days.

In some ~~embodiment~~examples, a plurality of T cells is exposed to a medium. In further ~~embodiment~~examples, the plurality of T cells is obtained from one or more source subjects before exposing to the medium.

In some ~~embodiment~~examples, the method further comprises isolating the T cell, or one or more progeny T cells thereof, from the medium after exposure. In some ~~embodiment~~examples wherein a population of T cells (e.g., T_N cells) have been exposed, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% of the population of the T cells (e.g., T_N cells) or one or more progeny cells thereof after the exposure are T cells having the characteristics of CD62L+ and CD45RO-.

In some ~~embodiment~~examples, the method further comprises administering the T cell (or T cells), or one or more progeny T cells thereof, to a subject in need thereof.

In some ~~embodiment~~examples, the method further comprises transducing the cell (or T cells, e.g., T_N cells) with a heterologous nucleic acid molecule comprising a sequence that encodes an immune receptor. In some ~~embodiment~~examples, the immune receptor is an antigen receptor that comprises an extracellular domain that specifically binds to an antigen of interest, wherein the extracellular domain is operatively linked to an intracellular domain that activates the T cell upon binding of the extracellular domain to the antigen of

interest. In some ~~embodiment~~examples, the immune receptor is a T cell receptor (TCR) that specifically binds to a peptide of interest bound to a major histocompatibility complex (MHC) molecule.

In another ~~aspect~~example, the disclosure provides an *in vitro* method of generating a T cell expressing a heterologous immune receptor. The method comprises performing the culturing method described herein; and, during the exposing step, transducing the T cell with a heterologous nucleic acid molecule comprising a sequence that encodes an immune receptor.

In some ~~embodiment~~examples, the T cell is a naïve T cell (T_N).

In some ~~embodiment~~examples, the immune receptor comprises an extracellular domain that specifically binds to an antigen of interest, wherein the extracellular domain is operatively linked to an intracellular domain that activates the T cell upon binding of the extracellular domain to the antigen of interest. In some ~~embodiment~~examples, the immune receptor is a T cell receptor (TCR) that specifically binds to a peptide of interest bound to a major histocompatibility complex (MHC) molecule. In some ~~embodiment~~examples, the method further comprises administering the T cell, or one or more progeny T cells thereof, to a subject in need thereof.

In another ~~aspect~~example, the disclosure provides a method of adoptive cell therapy. The method comprises administering a therapeutically effective number of cells produced by the *in vitro* methods of culturing a T cell or T cell population, described herein, to a subject in need thereof. In some ~~embodiment~~examples, the cells are produced by *in vitro* methods of culturing a naïve T cell (T_N), or a population thereof, as described herein. In some ~~embodiment~~examples, the subject has a condition selected from cancer, infectious disease, and autoimmune disease.

Regarding any methodological ~~aspect~~example described herein, in some ~~embodiment~~examples the T cell or population of T cells express a chimeric antigen receptor. In some further ~~embodiment~~examples, T cell is a naïve T cell (T_N) or the population of T cells comprise T_N as described.

In another ~~aspect~~example, the disclosure provides a cell were population cells produced by any one of the methods described herein.

In another ~~aspect~~example, the disclosure provides a therapeutic composition comprising a plurality of cells, as described herein, and an effective carrier.

In another ~~aspect~~example, the disclosure provides a method of reducing or preventing exhaustion of a T_N cell expressing a chimeric antigen receptor or the population of T_N cells expressing a chimeric antigen receptor. The method comprises exposing the T_N cell or the population of T_N cells to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell.

In another ~~aspect~~example, the disclosure provides a method of generating a T_N cell expressing a chimeric antigen receptor or a population of T_N cells expressing a chimeric antigen receptor. The method comprises modifying the T_N cell or the population of T_N cells to express a chimeric antigen receptor to a medium comprising a Notch receptor agonist, wherein the Notch receptor agonist reduces or prevents exhaustion of the T_N cell.

Regarding any method described herein, in some ~~embodiment~~examples the Notch receptor agonist is not expressed on any cell being co-cultured with the T cell(s). For example, in some ~~embodiment~~examples, the Notch receptor agonist is not expressed on by APC cells or bone marrow cells (e.g., OP9-DL1 cells). In some ~~embodiment~~examples of the methods described herein, the T cell or the population of T cells is not co-cultured with APCs. In some ~~embodiment~~examples of the methods described herein, the T cell or the population of T cells is not co-cultured with bone marrow cells. In some ~~embodiment~~examples of the methods described herein, the T cell or the population of T cells is not co-cultured with OP9-DL1 cells. In some ~~embodiment~~examples of the methods described herein, the T cell is a T_N cell, or the population of T cells is a population of T_N cells, and the T cell population thereof is not co-cultured with OP9-DL1 cells. In some ~~embodiment~~examples of the methods described herein, the T cell is a T_N cell, or the population of T cells is a population of T_N cells, and the T cell population thereof is not co-cultured with bone marrow cells. In some ~~embodiment~~examples of the methods described herein, the Notch receptor agonist is not DLL1.

In another ~~aspect~~example, the disclosure provides a method of culturing a T cell or T cells *in vitro*, comprising exposing a T cell or a population of T cells, to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell, wherein the Notch receptor agonist is a peptide ligand, and wherein the T cell is not co-cultured with an OP9-DL1 cell or a bone marrow cell expressing Delta-like 1 (DLL1). In some ~~embodiment~~examples, the T cell or the population of T cells exhibits at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 100%, at least about 110%, at least about 120%, at least about 130%, at least about 140%,

at least about 150%, at least about 160%, at least about 170%, at least about 180%, at least about 190%, at least about 200%, at least about 250%, or at least about 300% less exhaustion compared to a T cell co-cultured with OP9-DL1 cells.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 graphically illustrates the relative increase of Notch signaling (as determined by *Hes1* expression) of naïve T cells exposed to immobilized DLL1 (a Notch agonist) (μg/ml) over similar cells exposed to immobilized IgG1 (as control ligand, with irrelevant binding), or cells incubated in blank (no ligand) tissue culture ("TC"; as control). Cells were cultured for 4 hours on plates coated with DLL1-$^{Ext\ IgG}$ with retronectin, IgG1 with retronectin, or TC control plates. *Hes1* RNA was isolated, transcribed into cDNA, and quantified using SYBRgreen q-PCR. This culture method results in *Hes1* upregulation, showing Notch signaling.

FIGURES 2A and 2B graphically illustrate the relative expression of Notch1 and Notch2 receptors, respectively, in human CD4+ and CD8+ T cells at different time points before and after culture under different conditions. The cultures are indicated in the lower legend (TC = culture plates coated with retronectin only, DLL1 = is the Notch agonist for experimental condition, and IgG = culture plates coated with control IgG ligand; all plates coated with retronectin).

FIGURES 3A-3D graphically illustrate the relative expression of Notch ligands (Notch receptor agonists) DLL1, DLL4, JAG1, and JAG2, respectively, in human CD4+ and CD8+ T cells at different time points before and after culture under different conditions. The cultures are indicated in the lower legend (TC = tissue culture, DLL1 = is the Notch agonist as the experimental condition, and IgG is the control).

FIGURE 4 illustrates gating strategy and cell markers used in exemplary flow cytometry assays to identify differentiated subsets of T cells.

FIGURES 5A-5E graphically illustrate relative proportions of differentiated subsets for human CD4+ or CD8+ T cells, as determined by flow cytometry after incubation with different concentrations Notch ligands DLL1, anti-Notch1 antibody (N1), anti-Notch2 antibody (N2), or DLL4. Prior to analysis, human naïve T cells were

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

~~1. A method of culturing a naïve T (T_N) cell *in vitro*, comprising exposing a naïve T (T_N) cell to a medium comprising Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell.~~

~~2. A method of culturing naïve T (T_N) cells *in vitro*, comprising exposing a population of naïve T (T_N) cells to a medium comprising Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell.~~

~~3~~1. A method of culturing naïve T (T_N) cells *in vitro*, comprising exposing a population of naïve T(T_N) cells to a medium comprising Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell~~The method of Claim 2~~, wherein the population of T_N cells comprises at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% of T_N cells.

~~4~~2. The method of Claim ~~2~~1, wherein the population comprises about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 80% to about 90%, about 40% to about 80%, about 50% to about 80%, about 60% to about 80%, about 70% to about 80%, about 40% to about 70%, about 50% to about 70%, about 60% to about 70%, about 40% to about 60%, about 50% to about 60%, or about 40% to about 50% of T_N cells.

~~5~~3. The method of any one of Claims 1-~~4~~2, wherein the T cell is further characterized as CD62L+, CD45RA+, CD45RO-, CD95-, and/or CCR7+.

~~6~~4. The method of any one of Claims ~~2~~ 1 to ~~5~~3, wherein the exposing lasts for a period ("the exposure time") of at least about 12 hours, at least about one day, at least about two days, at least about three days, at least about four days, at least about five days, at least

about six days, at least about a week, at least about eight days, at least about nine days, at least about ten days, at least about 11 days, at least about 12 days, at least about 13 days, at least about 14 days, at least about 15 days, at least about 16 days, at least about 17 days, at least about 18 days, at least about 19 days, at least about 20 days, at least about 21 days, at least about 22 days, at least about 23 days, at least about 24 days, at least about 25 days, at least about 26 days, at least about 27 days, at least about 28 days, at least about 29 days, at least about 30 days, or at least about a month.

~~7~~5. The method of Claim ~~6~~4, wherein the exposure time is between one day to 15 days or between two days to 10 days.

~~8~~6. The method of any one of Claims ~~2~~ 1 to ~~7~~5, wherein the percentage of the T_N cells in the population does not change after the exposing.

~~9~~7. The method of any one of Claims ~~2~~ 1 to ~~7~~5, wherein the percentage of the T_N cells in the population changes, after the exposing, by less than about 1%, less than about 2%, less than about 5%, less than about 10%, less than about 15%, less than about 20%, less than about 25%, less than about 30%, less than about 35%, less than about 40%, less than about 45%, or less than about 50%.

~~10~~8. The method of any one of Claims ~~2~~ 1 to ~~9~~7, wherein the percentage of the T_N cells in the population is: (i) at least about 40% before the exposing and at least about 40% after the exposing; (ii) at least about 50% before the exposing and at least about 40% after the exposing; (iii) at least about 50% before the exposing and at least about 50% after the exposing; (iv) at least about 50% before the exposing and at least about 60% after the exposing; (v) at least about 60% before the exposing and at least about 50% after the exposing; (vi) at least about 60% before the exposing and at least about 60% after the exposing; (vii) at least about 60% before the exposing and at least about 70% after the exposing; (viii) at least about 70% before the exposing and at least about 60% after the exposing; (ix) at least about 70% before the exposing and at least about 70% after the exposing; (x) at least about 70% before the exposing and at least about 80% after the exposing; (xi) at least about 80% before the exposing and at least about 70% after the

exposing; (xii) at least about 80% before the exposing and at least about 80% after the exposing; (xiii) at least about 80% before the exposing and at least about 90% after the exposing; (xiv) at least about 90% before the exposing and at least about 80% after the exposing; (xv) at least about 90% before the exposing and at least about 90% after the exposing; or (xvi) at least about 90% before the exposing and about 100% after the exposing.

~~11~~9. The method of any one of Claims 1 to ~~10~~8, wherein the T_N cell, the population of T_N cells, or one or more progeny cells thereof, maintains at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.6 fold, at least 1.7 fold, at least 1.8 fold, at least 1.9 fold, at least 2.0 fold, at least 2.2 fold, at least 2.3 fold, at least 2.4 fold, at least 2.5 fold, at least 2.6 fold, at least 2.7 fold, at least 2.8 fold, at least 2.9 fold, at least 3 fold, at least 3.5 fold, at least 4.0 fold, at least 4.5 fold, at least 5.0 fold, at least 5.5 fold, at least 6.0 fold, at least 6.5 fold, or at least 7.0 fold less-differentiated state *in vivo* compared to a T_N cell that did not receive the Notch receptor agonist.

~~12~~10. The method of any one of Claims 1 to ~~11~~9, wherein the Notch receptor agonist comprises a domain of a mammalian Notch receptor ligand that binds to a mammalian Notch1, Notch2, Notch3, or Notch4 receptor.

~~13. The method of any one of Claims 1 to 12, wherein the Notch receptor agonist is or comprises a Delta protein, a Jagged protein, an anti-Notch antibody, or a fragment or derivative thereof, that binds to a mammalian Notch receptor, or any combination thereof.~~

~~14. The method of any one of Claims 1 to 13, wherein the Notch receptor agonist, upon binding to a Notch receptor, is capable of inducing a conformational change of the Notch Receptor.~~

~~15~~11. The method of any one of Claims 1 to ~~14~~10, wherein the Notch receptor agonist, upon binding to a Notch receptor, is capable of exposing an S2 cleavage site in the negative regulatory region (NRR) of the Notch receptor.

~~16. The method of any one of Claims 13 to 15, wherein the Notch receptor agonist comprises an extracellular domain of a Delta protein or a Jagged protein.~~

~~17. The method of Claim 13, wherein the Notch receptor agonist is or comprises Delta Like Ligand 1 (DLL1), Delta Like Ligand 3 (DLL3), Delta Like Ligand 4 (DLL4), Jagged1, Jagged2, Dlk1, Dlk2, DNER, EGFL 7, F3/contactin, a fragment thereof, a derivative thereof, or any combination thereof.~~

~~18~~12. The method of any one of claims 1 to 10~~Claim 17~~, wherein the Notch receptor agonist is or comprises DLL1, DLL3, and/or DLL4.

~~19. The method of Claim 17, wherein the Notch receptor agonist is or comprises Jagged1 and/or Jagged2.~~

~~20~~13. The method of any one of claims 1 to 10~~Claim 13~~, wherein the Notch receptor agonist is or comprises an anti-Notch antibody or an antigen binding fragment thereof.

~~21~~14. The method of Claim ~~20~~13, wherein the anti-Notch antibody or an antigen binding fragment thereof binds to an epitope in the Notch extracellular domain (NECD) that is not in the negative regulatory region (NRR) of the Notch receptor.

~~22~~15. The method of claim ~~20~~13, wherein the anti-Notch antibody binds to Notch1, Notch2, Notch3, and/or Notch4.

~~23~~16. The method of any one of Claims 1 to ~~22~~15, wherein the Notch receptor agonist is present at a concentration of about 0.01 µg/ml to about 100 µg/ml.

~~24~~17. The method of any one of Claims 1 to ~~23~~16, wherein the Notch receptor agonist is immobilized on a surface or scaffold.

~~25~~18. The method of Claim ~~23~~16, wherein the T_N cell or the population of T_N cells is exposed to the medium at a concentration sufficient to contact substantially all of the immobilized Notch receptor agonist.

~~26~~19. The method of any one of Claims 1 to ~~25~~18, wherein the medium further comprises one or more cytokines, or a biologically active fragment thereof, that modulate T_N cell differentiation.

~~27. The method of Claim 26, wherein the one or more cytokines include IL-1, IL-1b, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-15, IL-17, IL-21, IL-23, IL-27, IFN γ, TNF α, TGFβ, or any combination thereof, in an effective concentration.~~

~~28. The method of any one of Claim 1 to 27, wherein the T_N cell or the population of the T_N cells is obtained from one or more source subjects before being exposed to the medium.~~

~~29. The method of any one of Claims 1 to 28, further comprising isolating the T_N cell, the population of T_N cells, or one or more progeny cells thereof, from the medium after the exposure.~~

~~30~~20. The method of any one of claims 1 to 19~~Claim 29~~, wherein at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% of the population of the T_N cells or one or more progeny cells thereof after the exposure are T cells having the characteristics of CD62L+ and CD45RO-.

~~31~~21. The method of any one of Claims 1 to ~~30~~20, further comprising transducing the T_N cell, the population of T_N cells, or one or more progeny cells thereof with a heterologous nucleic acid molecule comprising a sequence that encodes an immune receptor.

~~32~~22. The method of Claim ~~31~~21, wherein the immune receptor is an antigen receptor that comprises an extracellular domain that specifically binds to an antigen of interest, wherein the extracellular domain is operatively linked to an intracellular domain that activates the T cell upon binding of the extracellular domain to the antigen of interest.

~~33~~23. The method of Claim ~~31~~21, wherein the immune receptor is a T cell receptor (TCR) that specifically binds to a peptide of interest bound to a major histocompatibility complex (MHC) molecule.

~~34~~24. The method of any one of Claims ~~29~~ 20 to ~~33~~23, further comprising ~~administering~~ the T_N cell, the population of T_N cells, or one or more progeny cells thereof, to be administered to a subject in need thereof.

~~35~~25. An *in vitro* method of generating a T_N cell expressing a heterologous immune receptor, comprising:

performing the method recited in any one of Claims 1 to ~~30~~24; and

during the exposing step, transducing the T_N cell with a heterologous nucleic acid molecule comprising a sequence that encodes an immune receptor.

~~36~~26. The method of Claim ~~35~~25, wherein the immune receptor comprises an extracellular domain that specifically binds to an antigen of interest, wherein the extracellular domain is operatively linked to an intracellular domain that activates the T cell upon binding of the extracellular domain to the antigen of interest.

~~37~~27. The method of Claim ~~35~~25, wherein the immune receptor is a T cell receptor (TCR) that specifically binds to a peptide of interest bound to a major histocompatibility complex (MHC) molecule.

~~38~~28. The method of one of Claims ~~35~~ 25 to ~~37~~27, further comprising ~~administering~~ the T cell, or one or more progeny T cells thereof, to be administered to a subject in need thereof.

~~39. A method of adoptive cell therapy, comprising administering a therapeutically effective number of cells produced by the method recited in any one of Claims 1 to 30 and 32-37 to a subject in need thereof.~~

~~40. The method of Claim 39, wherein the subject has a condition selected from cancer, infectious disease, and autoimmune disease.~~

~~41. A T cell produced by any one of the methods of Claims 1 to 33 and 35 to 37.~~

~~42. A therapeutic composition comprising a plurality of cells recited in Claim 41 and an effective carrier.~~

~~43. The method of any one of Claims 1 to 40, wherein the T_N cell or the population of T_N cells expresses a chimeric antigen receptor.~~

~~44~~29. A method of reducing or preventing exhaustion of a T_N cell expressing a chimeric antigen receptor or of a population of T_N cells expressing a chimeric antigen receptor, the method comprising exposing the T_N cell or the population of T_N cells to a medium comprising a Notch receptor agonist for a time sufficient to induce Notch receptor signaling in the cell.

~~45~~30. A method of generating a T_N cell or a population of T_N cells expressing a chimeric antigen receptor, the method comprising modifying the T_N cell or the population of T_N cells to express a chimeric antigen receptor to a medium comprising a Notch receptor agonist, wherein the Notch receptor agonist reduces or prevents exhaustion of the T_N cell.

~~46~~31. The method of any one of Claims 1 to ~~40 and 43 to 49~~30, wherein the Notch receptor agonist is an anti-Notch antibody.