

Claims

1. A method of expanding activated T cells to produce T cells with enhanced in vivo persistence after adoptive transfer, comprising contacting a cell population comprising activated T cells with an effective amount of a compound that uncouples T cell expansion from differentiation, thereby expanding the activated T cells to produce T cells with enhanced in vivo persistence after adoptive transfer.
2. The method of claim 1, wherein the activated T cells are stem memory T cells (Tscm), central memory T cells (Tcm), effector memory T cells, effector T cells, progenitor exhausted T cells (Tpex), or terminally exhausted T cells (Ttex).
3. The method of claim 1, wherein the produced T cells are less differentiated relative to the activated T cells not treated with the compound.
4. The method of claim 1, wherein the compound is a sugar or sugar derivative.
5. The method of claim 4, wherein the sugar is trehalose, sucrose, lactose, fructose or neuraminic acid.
6. The method of claim 4, wherein the sugar derivative is *N*-Acetylglucosamine (GlcNAc) or *N*-acetylneuraminic acid (Neu5Ac).
7. The method of claim 1, wherein the compound is an inhibitor of Enhancer of zeste homolog 2 (EZH2).
8. The method of claim 7, wherein the inhibitor is Tazemetostat.
9. The method of claim 1, wherein the compound is a small molecule inhibitor of KRAS (G12C) mutant.
10. The method of claim 9, wherein the inhibitor is KRAS (G12C) inhibitor 12.
11. The method of claim 1, wherein the compound is guanosine, 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), or Glucose-6-phosphate Dehydrogenase Inhibitor 1 (G6PDi-1).
12. The method of claim 1, wherein the T cell is a tumor infiltrating lymphocyte (TIL) or a genetically engineered T cell.
13. The method of claim 12, wherein the TIL is a CD8⁺ T cell or a CD4⁺ T cell.
14. The method of claim 12, wherein the genetically engineered T cell is a TCR-modified T cell or a CAR-T cell.
15. A method for converting a TCF-1 negative T cell into partially TCF-1 positive, comprising contacting a population of TCF-1 negative T cells with an effective amount of a

compound that uncouples T cell expansion from differentiation, thereby converting the TCF-1 negative T cell into partially TCF-1 positive.

16. The method of claim 15, wherein the compound is *N*-acetylneuraminic acid (Neu5Ac) or *N*-Acetylglucosamine (GlcNAc).

17. A kit for in vitro expansion of an activated T cell for adoptive cell therapy, comprising (1) an effective amount of a compound that uncouples T cell expansion from differentiation, and (2) an instruction of co-culturing the compound with a population of cells comprising the activated T cell.

18. The kit of claim 17, wherein the activated T cell is stem memory T cell (Tscm), central memory T cell (Tcm), effector memory T cell, effector T cell, progenitor exhausted T cell (Tpex), or terminally exhausted T cell (Ttex).

19. A method of screening for compounds that promote proliferation of activated T cells and/or maintain expanded T cells in a less differentiated state, comprising:

(a) providing T cells from a T cell-containing tissue from a subject;

(b) culturing activated T cells from the T cell-containing tissue in the presence of a compound; maintaining said culturing for a time period sufficient to permit proliferation of T cells, wherein T cells from the T cell-containing tissue have undergone stimulating by an activating agent, wherein stimulating occurs prior to or concurrently with culturing; and

(c) measuring: (i) an amount of T cells expressing TCF-1; and/or (ii) an amount of TCF-1 expression, wherein an increase in both (i) and (ii) relative to T cells after expansion not treated with the compound identifies the compound as promoting proliferation of progenitor exhausted T cells and maintaining expanded T cells in a less differentiated state, and wherein an increase in (ii) and not (i) relative to T cells after expansion not treated with the compound identifies the compound as promoting maintaining expanded T cells in a less differentiated state.

20. The method of claim 19, further comprising re-stimulating and expanding the T cells in the culture following (c), and maintaining said culturing for a time period sufficient to permit expansion of T cells, wherein T cells are assessed by measuring: (i) an amount of T cells expressing TCF-1; and/or (ii) an amount of TCF-1 expression, wherein an increase in both (i) and (ii) relative to T cells after expansion not treated with the compound identifies the compound as promoting proliferation of progenitor exhausted T cells and maintaining expanded T cells in a less differentiated state, and wherein an increase in (ii) and not (i) relative to T cells after expansion not treated with the compound identifies the compound as promoting maintaining expanded T cells in a less differentiated state.