

To date, clinical efficacy of engineered T cell adoptive immunotherapies is limited by poor T cell expansion and persistence after infusion into patients. Therefore, such therapies are not suitable for widespread clinical use. Accordingly, there is a persistent, unmet need for improvements in T cell manufacturing and therapeutic T cell compositions that survive, expand, and persist *in vivo*.

SUMMARY

The disclosure generally provides adoptive T cell immunotherapies comprising persistent and potent anti-tumor T cell compositions and methods of making the same.

The present disclosure relates to improved T cell compositions and methods for manufacturing T cells. More particularly, the disclosure relates to methods of T cell manufacturing that result in with improved survival, expansion, and persistence *in vivo*.

In various embodiments, a method for manufacturing T cells comprising: (a) isolating a population of T cells, e.g., tumor infiltrating cytotoxic T lymphocytes (TIL), from a subject; (b) activating the population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of an inhibitor of AKT/mTOR pathway; (c) culturing the T cells to proliferate; wherein the activating and stimulating steps performed in the presence of the inhibitor of the PI3K/AKT/mTOR pathway results in maintaining proliferation of the T cells compared to the proliferation of T cells that were activated and stimulated in the absence of the inhibitor of the PI3K/AKT/mTOR pathway is provided.

In various embodiments, a method for manufacturing T cells comprising: (a) activating a population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of an inhibitor of AKT/mTOR pathway; (b) transducing the T cells with a viral vector comprising an engineered T cell receptor (TCR) or a chimeric antigen receptor (CAR); (c) culturing the transduced T cells to proliferate; wherein the activating and stimulating steps performed in the presence of the inhibitor of the PI3K/AKT/mTOR pathway results in maintaining proliferation of the transduced T cells compared to the proliferation of transduced T cells that were activated and stimulated in the absence of the inhibitor of the PI3K/AKT/mTOR pathway is provided.

In a first aspect, there is provided an *in vitro* or *ex vivo* method for manufacturing T cells comprising: (a) activating a population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of a PI3K inhibitor; (b) transducing the T cells with a viral vector comprising an engineered T cell receptor (TCR) or a chimeric antigen receptor (CAR); (c) culturing the transduced T cells to proliferate; wherein the activating and stimulating steps performed in the presence of the PI3K inhibitor results in maintaining proliferation of the transduced T cells compared to the proliferation of transduced T cells that were activated and stimulated in the absence of the PI3K inhibitor.

CLAIMS

1. An *in vitro* or *ex vivo* method for manufacturing T cells comprising:

(a) activating a population of T cells and stimulating the population of T cells to proliferate, wherein the activation and stimulation steps are performed in the presence of a PI3K inhibitor;

(b) transducing the T cells with a viral vector comprising an engineered T cell receptor (TCR) or a chimeric antigen receptor (CAR); and

(c) culturing the transduced T cells to proliferate;

wherein the activating and stimulating steps performed in the presence of the PI3K inhibitor results in maintaining proliferation and decreasing differentiation of the transduced T cells compared to the proliferation and differentiation of transduced T cells that were activated and stimulated in the absence of the PI3K inhibitor.

2. The method of claim 1, wherein

(a) the source of T cells is peripheral blood mononuclear cells;

(b) the activation of the T cells comprises contacting the T cells with an anti-CD3 antibody or CD3-binding fragment thereof;

(c) the stimulation of the T cells comprises contacting the T cells with an anti-CD28 antibody or a CD28-binding fragment thereof, B7-1 or a CD28-binding fragment thereof, or B7-2 or a CD28-binding fragment thereof;

(d) the T cells are transduced with the viral vector prior to T cell proliferation; or

(e) the T cells are transduced with the viral vector after T cell proliferation.

3. The method of any one of claims 1 to 2, wherein:

(a) the viral vector is a retroviral vector;

(b) the viral vector is a lentiviral vector; or

(c) the T cells comprise a CAR.

4. The method of claim 3, wherein the CAR comprises

a) an extracellular domain that binds an antigen selected from the group consisting of: BCMA, alpha folate receptor, 5T4, $\alpha_v\beta_6$ integrin, B7-H3, B7-H6, CAIX, CD19, CD20, CD22, CD30, CD33, CD44, CD44v6, CD44v7/8, CD70, CD79a, CD79b, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EGP2, EGP40, EPCAM, EphA2, EpCAM, FAP, fetal AchR, FR α , GD2, GD3, Glypican-3 (GPC3), HLA-A1+MAGE1, HLA-A2+MAGE1, HLA-A3+MAGE1, HLA-A1+NY-ESO-1, HLA-A2+NY-ESO-1, HLA-A3+NY-ESO-1, IL-11R α , IL-13R α 2, Lambda, Lewis-Y, Kappa, Mesothelin, Muc1, Muc16, NCAM, NKG2D Ligands, NY-ESO-1, PRAME, PSCA, PSMA, ROR1, SSX, Survivin, TAG72, TEMs, and VEGFR2;

b) a transmembrane domain derived from a polypeptide selected from the group consisting of: CD8 α ; CD4, CD28, CD45, PD-1, and CD152;

c) one or more intracellular costimulatory signaling domains selected from the group consisting of: CD28, CD54 (ICAM), CD134 (OX40), CD137 (41BB), CD152 (CTLA4), CD273 (PD-L2), CD274 (PD-L1), and CD278 (ICOS); and

d) a CD3 ζ signaling domain.

5. The method of claim 4, wherein:

(a) the extracellular domain comprises an antibody or antigen binding fragment that binds the antigen;

(b) the transmembrane domain is derived from CD8 α or CD28;

(c) the one or more costimulatory signaling domains selected from the group consisting of: CD28, CD134, and CD137;

(d) the CAR further comprises a hinge region polypeptide;

(e) the CAR further comprises a hinge region polypeptide that comprises a hinge region of IgG1 or CD8 α ;

(f) the CAR further comprises a signal peptide;

(g) the CAR further comprises a signal peptide that comprises an IgG1 heavy chain signal polypeptide or a CD8 α signal polypeptide.

6. The method of any one of claims 1 to 5, wherein the PI3K inhibitor is:

(a) a pan-PI3K inhibitor selected from the group consisting of: BEZ235, LY294002, and GDC-0941;

(b) a selective PI3K inhibitor selected from the group consisting of: BYL719, GSK2636771, TGX-221, AS25242, CAL-101, and IPI-145; or

(c) ZSTK474.

7. The method of any one of claims 1 to 6, wherein the population of T cells activated and stimulated in the presence of a PI3K inhibitor have an increased number of T cells expressing one or more markers selected from the group consisting of: CD62L, CCR7, CD28, CD27, CD122, and CD127 compared to a population of T cells activated and stimulated in the absence of the PI3K inhibitor.

8. The method of any one of claim 7, wherein the population of T cells activated and stimulated in the presence of a PI3K inhibitor do not express CD57 or KLRG1 or express less CD57 or KLRG1 compared to a population of T cells activated and stimulated in the absence of the PI3K inhibitor.