

CLAIMS

1. A method of expanding and/or activating a population of immune cells, e.g., immune effector cells, comprising:

introducing a nucleic acid encoding a first Chimeric Antigen Receptor (CAR) molecule into the immune cell population, under conditions suitable for transient expression of the CAR molecule, thereby producing a first CAR-expressing cell population, wherein the CAR molecule comprises an antigen binding domain of an antibody molecule; and

contacting the first CAR-expressing cell population with an anti-antigen idiotypic antibody molecule under conditions such that immune cell expansion and/or activation occurs, thereby producing an expanded and/or activated immune cell population; wherein:

(i) the expansion and/or activation of the population of immune cells is carried out *in vitro* or *ex vivo*; and/or

(ii) the population of immune cells is acquired, *e.g.*, obtained, from a blood sample from a subject (e.g., a cancer patient), *e.g.*, by apheresis.

2. A method of expanding and/or activating a population of immune cells, e.g., immune effector cells, comprising:

providing a first CAR-expressing cell population, said first CAR-expressing cell population comprising a transiently expressed first CAR molecule, and said CAR molecule comprises an antigen binding domain of an antibody molecule; and

contacting said first CAR-expressing cell population with an anti-antigen idiotypic antibody molecule that binds to said CAR under conditions such that immune cell expansion and/or activation occurs, thereby producing an expanded and/or activated immune cell population; wherein:

(i) the expansion and/or activation of the population of immune cells is carried out *in vitro* or *ex vivo*; and/or

(ii) the population of immune cells is acquired, *e.g.*, obtained, from a blood sample from a subject (e.g., a cancer patient), *e.g.*, by apheresis.

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

(i) the population of immune cells comprises immune effector cells chosen from T cells (e.g., alpha/beta T cells and gamma/delta T cells), B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, myeloid-derived phagocytes, or a combination thereof;

(ii) the population of immune cells comprises primary T cells or a subset of lymphocytes chosen from anergized T cells, naïve T cells, T-regulatory cells, Th-17 cells, stem T cells, or a combination thereof;

(iii) the population of immune cells comprises peripheral blood mononucleated cells (PBMCs), cord blood cells, or a combination thereof; and/or

(iv) the population of immune cells comprises cells that do not have a functional T cell receptor and/or that express a mutated or truncated form of one or more of a subunit of a T cell receptor.

4. The method of any of claims 1-3, wherein the anti-antigen idiotypic antibody molecule is:

immobilized or attached to a non-naturally occurring substrate, *e.g.*, a substrate surface, optionally wherein the non-naturally occurring substrate is a solid support chosen from a plate, a membrane, a matrix, a chip or a bead (*e.g.*, a magnetic bead).

5. The method of any of claims 1-4, wherein the nucleic acid encoding the first CAR molecule is an RNA molecule, *e.g.*, an *in vitro* transcribed (IVT) RNA.

6. The method of any of claims 1-5, wherein:

(i) the first CAR molecule is transiently expressed in the immune cell population for a finite period of time or number of cell replications, *e.g.*, less than 50 days (*e.g.*, less than 40, 30, 25, 20, 15, 10, 5 or fewer days);

(ii) the first CAR molecule is internalized post a stimulation by a single anti-antigen idiotypic antibody molecule;

(iii) the immune cell does not receive repeated anti-antigen idiotypic antibody molecule stimulation;

(iv) the strength of the immune cell stimulation is customized to a desired level by adjusting one or both of: the first CAR-surface density, or the affinity of the CAR antigen binding domain to the anti-antigen idiotypic antibody molecule;

(v) the first CAR-expressing immune cells are cultured in the presence of the anti-antigen idiotypic antibody molecule of the CAR molecule for about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18, 21, 22, 23 or 24 hours, or about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 days, optionally wherein the first CAR-expressing cells are cultured for a period of 8 days or less, *e.g.*, 7, 6 or 5 days.

(vi) the CAR-expressing cells shows at least 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 or higher population doublings (*e.g.*, a total of 8-10, or about 9 population doublings), optionally wherein the first CAR-expressing immune cell population expands to a total of 400-600, or about 500 cells, wherein the cell expansion is measured between 10 and 25 days after stimulation with the anti-antigen idiotypic antibody molecule; and/or

(vii) the expanded and/or activated immune cell population comprises immune effector cells having a less differentiated phenotype, *e.g.*, a younger cell, *e.g.*, a younger T cell, optionally wherein the younger T cell is chosen from a naïve T cell (T_N), a memory stem cell (T_{SCM}), a central memory T cell (T_{CM}), or a combination thereof.

7. Use of an expanded and/or activated immune cell population made according to one or more of claims 1-6 in the manufacture of a medicament for the treatment of a cancer, or provision of an anti-tumor immunity, in a subject, wherein the expanded and/or activated immune cell population, is/are to be administered alone or in combination with an additional therapeutic agent.
8. An expanded and/or activated immune cell population that expresses a first CAR molecule made according to one or more of claims 1-6 for use in a method of treating, or providing anti-tumor immunity to, a subject having a cancer, wherein said expanded and/or activated immune cell population is to be administered to the subject alone or in combination with an additional therapeutic agent.
9. The method or use of any one of the preceding claims, wherein:
- (i) the first CAR-expressing immune effector cell comprises a CD19 CAR, a BCMA CAR, a CD33 CAR, a CLL-1 CAR, EGFRvIII CAR, a GFR alpha 4 CAR, an ROR1 CAR, a CD19 CAR, a CD20 CAR, a CD22 CAR, a CD123 CAR, a CD10 CAR, a CD34 CAR, a FLT-3 CAR, a CD79b CAR, a CD179b CAR, a mesothelin CAR or a CD79a CAR;
 - (ii) first CAR-expressing immune effector cell comprises a CD19 CAR;
 - (iii) the CD19 CAR comprises a sequence according to Table 1 or Table 4;
 - (iv) the CD19 CAR comprises the amino acid sequence of the antigen binding domain of CTL019, e.g., SEQ ID NO: 51, or an amino acid sequence substantially identical thereto;
 - (v) the CD19 CAR comprises the amino acid sequence of CTL019, e.g., SEQ ID NO: 89 (including the signal sequence) or without the signal sequence, or an amino acid sequence substantially identical thereto;
 - (vi) at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96 %, 97%, 98%, 99% or 100% of the immune effector cells in the immune effector cell population express the first and/or the second CAR molecule on their cell surface; or
 - (vii) the subject from which immune effector cells are acquired and/or the subject to be treated, is a human cancer patient.
10. The method or use of any one of the preceding claims, wherein the cancer is a hematological cancer chosen from one or more of: a B-cell acute lymphocytic leukemia (B-ALL), T-cell acute lymphocytic leukemia (T-ALL), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), B cell promyelocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma (MCL), marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma (NHL),

Hodgkin's lymphoma (HL), plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, and Waldenstrom macroglobulinemia.

11. The method or use of any one of the preceding claims, wherein the population of immune effector cells are from a subject having a haematological cancer chosen from a leukemia, e.g., chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), or a lymphoma, e.g., mantle cell lymphoma (MCL).

12. The method or use of any of the preceding claims, wherein the population of cells is expanded in the presence a cytokine, e.g., IL2 or IL-15 and IL-7.

13. The method or use of any one of claims 1-12, wherein the acquired immune effector cell population has been selected based upon the expression of one or more markers, e.g., CD3, CD28, CD4, CD8, CD45RA, and CD45RO, e.g., the provided population of immune effector cells (e.g., T cells) are CD3⁺ and/or CD28⁺.

14. An immune cell preparation or reaction mixture, comprising a population of immune effector cells made according to any of the methods described herein.