CLAIMS

What is claimed is:

1. A method of producing an immune cell culture in a fully closed system, comprising:
    a. obtaining an immune cell;
    b. introducing the immune cell into a stirred-tank bioreactor comprising an immune cell complete medium;
    c. activating the immune cell with an activation reagent to produce an activated immune cell in the stirred-tank bioreactor;
    d. expanding the activated immune cell to produce an expanded immune cell culture in the stirred-tank bioreactor;
    e. exchanging a defined amount of fresh medium for spent medium via an alternating tangential flow filtration (ATF) connected to the bioreactor;
    f. depleting the expanded immune cell culture of (d) to produce a depleted immune cell culture in the stirred-tank bioreactor;
    g. harvesting the depleted immune cell culture of (f) to produce a harvested immune cell culture in the fully closed system; and
    h. concentrating the harvested immune cell culture of (g) in the fully closed system,
    i. wherein the method results in less than 1% loss of the immune cell culture.
2. The method of claim 1, wherein the immune cell complete medium comprises a buffer, amino acids, trace elements, vitamins, inorganic salts, glucose and serum.
3. The method of claim 1, wherein the immune cell is i) derived from a population of pluripotent stem cells, ii) isolated from a population of peripheral blood mononuclear cells (PBMCs) immediately prior to obtaining the immune cell in a) or iii) isolated from a population of PBMCs, stored for an extended period of time, and then thawed prior to obtaining the immune cell in a).
4. The method of claim 3, wherein isolating the immune cell comprises a) washing the PBMCs with a solution comprising antibody complexes and magnetic particles to produce a solution comprising the PBMCs and isolated T-cells and b) separating the isolated T-cells from the solution with a magnet.
5. The method of any one of the preceding claims, wherein between 0.25 $x10^6$ T-cells/mL and 2 x 10^6 T-cells/mL are introduced into the bioreactor.

6. The method of any one of the preceding claims, wherein the method produces an immune cell culture comprising about 10 x 10^6 cells/mL to about 90 x 10^6 cells/mL viable immune cells.
7. The method of any one of the preceding claims, wherein the activation reagent comprises soluble antibody complexes.
8. The method of any one of the preceding claims, wherein activating the immune cell comprises stirring the medium with the activation reagent for a period of about 72 hours at 37°C.
9. The method of any one of the preceding claims, wherein the medium has a pH between about pH 5.0 and about pH 7.5 during the activation period.
10. The method of any one of the preceding claims, wherein the expanding comprises:

    i. adding fresh medium to the bioreactor after the activation period in (c);
    ii. monitoring one or more of a temperature sensor, a pH sensor, a glucose sensor, an oxygen sensor, a carbon dioxide sensor, and an optical density sensor of the immune cell culture; and
    iii. adjusting one or more of a temperature, a pH level, a glucose level, an oxygen level, a carbon dioxide level, and an optical density of the immune cell culture, based on the monitoring.

11. The method of claim 10, wherein the expanding further comprises iv) sampling the expanding immune cell culture, v) determining a cell growth and fold expansion of the expanding T cell culture and vi) exchanging a defined amount of fresh medium for spent medium based on the cell growth and fold expansion.
12. The method of claim 11, wherein the fresh medium is exchanged for spent medium at a rate of one vessel volume per day (VVD) when the viable cell density of the expanding immune cell culture is greater than 1.5x10^6 cells/mL.
13. The method of any one of the preceding claims, wherein the depleting comprises:
    i) adding surface-activated magnetic beads to the immune cell culture after the expanding in d);
    ii) stirring the expanded immune cell culture and beads for about 30 minutes; and

iii) isolating a population of target cells from the expanded immune cell culture with a magnet.

14. The method of any one of the preceding claims, wherein the harvesting comprises i) pumping the immune cell culture from the stirred-thank bioreactor into a sterile container connected to the stirred-tank bioreactor and ii) pumping the immune cell culture from the ATF into the sterile container connected to a harvest line of the ATF.
15. The method of claim 14, wherein the connection between the stirred-tank bioreactor and the ATF is closed.
16. The method of claim 14, wherein the pumping of the cell culture from the stirred-tank bioreactor into the sterile container is performed while the immune cell culture is continuously stirred in the stirred-tank bioreactor.
17. The method of any one of the preceding claims, wherein the concentrating comprises centrifugation, supernatant removal following sedimentation, filtration, acoustic cell processing, or combinations thereof of the harvested cell culture.
18. The method of any one of the preceding claims, wherein the activating and the expanding of the immune cell in the stirred-tank bioreactor results in a T-cell culture producing greater than five cytokines, and with greater than 75% central memory T-cells, less than 10% effector memory T-cells, and greater than 10% naïve/stem memory T-cells.
19. A population of T-cells produced by a method according to any one of the preceding claims.
20. A T-cell produced by the method of any one of the preceding claims for use in a T-cell based therapy.