

CLAIMS

1. A method of assaying the potency of an immune cell, comprising contacting an immune cell with an effective amount of a stimulating agent and detecting the amount of a cytokine produced by the immune cell.
2. The method of claim 1, further comprising the step of comparing the amount of cytokine produced to the cytokine potency level required for use of the immune cell in immunotherapy.
3. The method of any of claims 1-3, wherein the amount of a plurality of cytokines is determined.
4. The method of any of claims 1-4, wherein the immune cell is a T-cell, a macrophage, a Natural Killer (NK) cell, NK T cell, chimeric antigen receptor (CAR) T cell, or CAR NK cell.
5. The method of claim 4, wherein the immune cell is an NK cell.
6. The method of claim 5, wherein the NK cell is an IL-21 expanded NK cell.
7. The method of any of claims 1-6, wherein the stimulating agent comprises phytohemagglutinin (PHA), phorbol myristate acetate (PMA)/ionomycin, concanavalin A (Con A), lipopolysaccharide (LPS), and/or pokeweed mitogen (PWM).
8. The method of any of claims 1-7, wherein the amount of cytokine is detected using an immunoassay.
9. The method of any of claims 1-8, wherein the cytokine is selected from the group comprising interleukin (IL)-2 (IL-2), IL-6, interferon (IFN)- γ (IFN- γ), B cell activating factor/ tumor necrosis factor (TNF) ligand superfamily member 13B (BAFF/TNFSF13B), TNF- α , cluster of differentiation (CD) 163 (CD163), CD30/TNFRSF8, Chitinase 3-like 1, gp130, IFN- α 2, IL-6Ra, IL-8, IL-10, IL-11, IL-12(p40), IL-12(p70), IL-20, IL-22, IL-26, IL-29/IFN-11, IL-32, IL-34, IL-35, matrix metalloproteinase-1 (MMP-1), Osteocalcin, Osteopontin (OPN), Pentraxin-3, tumor necrosis factor (TNF)- receptor 1 (TNF-R1), TNF-R2, thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), and the chemokines macrophage inflammatory protein (MIP) -1 α (MIP-1 α), MIP-1 β , RANTES, and/or TNF-related weak inducer of apoptosis (TWEAK)/TNF superfamily member 12 (TWEAK/TNFSF12).

10. The method of claim 1, wherein the immune cell is contacted with an effective amount of the stimulating agent for at least 4 hours.
11. The method of claim 1, wherein the stimulating agent is provided at a concentration of 5 $\mu\text{g/mL}$ to 15 $\mu\text{g/mL}$.
12. A kit for assaying the potency of an immune cell, comprising a container including an effective amount of a stimulating agent and a buffer suitable for immune cells.
13. The kit of claim 12, wherein the stimulating agent is provided at a concentration of 5 $\mu\text{g/mL}$ to 15 $\mu\text{g/mL}$.
14. The kit of claim 12, wherein the container is an Eppendorf microcentrifuge tube.
15. The kit of claim 12, wherein the kit further comprises instructions for using the kit to stimulate cytokine production by an immune cell.
16. A method comprising;
 - a. performing the method of any of claims 1-11 on multiple immune cells to determine the potency of each immune cell;
 - b. selecting at least one potent immune cell based on the amount of cytokine detected; and
 - c. preparing a therapeutically effective amount of the potent immune cell to be administered to a subject in need thereof as an immunotherapeutic.
17. The method of claim 16, further comprising extracting the multiple immune cells from an allogeneic or autologous donor prior to assaying the potency of the immune cell.
18. The method of claim 16, further comprising expanding the at least one potent immune cell prior to preparing a therapeutically effective amount of the potent immune cell.
19. The method of claim 16, further comprising directing the multiple immune cells or the potent immune cell to respond to a specified antigen.
20. The method of claim 19, further comprising genetically altering the multiple immune cells or the potent immune cell to present a chimeric antigen receptor.
21. Use of at least one potent immune cell obtained by the method of any one of claims 1-11 for the manufacture of a medicament for treating, inhibiting, reducing, preventing, and/or ameliorating a cancer and/or metastasis in a subject.

22. The use according to claim 21, wherein the immune cell is obtained from an allogeneic or autologous donor.
23. The use according to claim 21, wherein the method of any one of claims 1-11 further comprises extracting multiple immune cells from an allogeneic or autologous donor.
24. The use according to any of claims 21-23, wherein the immune cell is a T-cell, a macrophage, a Natural Killer (NK) cell, NK T cell, chimeric antigen receptor (CAR) T cell, or CAR NK cell.
25. The use according to any of claims 21-24, wherein the medicament comprises expanded potent immune cells.