be required to submit a substantial amount of information regarding the drug product to the regulatory authorities in order to obtain approval. This may include information regarding the potency of the drug product and assays to determine this potency. As required by the FDA (21 CFR 610.10), potency of the cell therapy product should be indicated by appropriate tests to show effector function of these therapeutic immune cells to show potency, which would be measuring relevant cytokine production by these immune cells.

SUMMARY

[0010] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

[0011] In one aspect, disclosed herein are methods of assaying the potency of an immune cell (such as, for example, a T-cell, a macrophage, a NK cell, NK T cell, CAR T cell, and/or CAR NK cell), comprising contacting an immune cell with an effective amount of a stimulating agent (such as, for example, phytohemagglutinin (PHA), phorbol myristate acetate (PMA)/ionomycin, concanavalin A (Con A), lipopolysaccharide (LPS), and/or pokeweed mitogen (PWM)) and detecting the amount of one or more cytokines (such as, for example, IL-2, IL-6, IFN-γ, TNF-α, BAFF/TNFSF13B, CD163, CD30/TNFRSF8, Chitinase 3-like 1, gp130, IFN-α2, IL-6Rα, IL-8, IL-10, IL-11, IL-12(p40), IL-12(p70), IL-20, IL-22, IL-26, IL-29/IFN-l1, IL-32, IL-34, IL-35, MMP-1, Osteocalcin, OPN, Pentraxin-3, TNF-R1, TNF-R2, TSLP, GM-CSF, MIP-1α, MIP-1β, RANTES, and/or TWEAK/TNFSF12) produced by the immune cell. In one aspect, the method can further comprise comparing the amount of cytokine produced to the cytokine potency level required for use of the immune cell in immunotherapy.

[0012] Also disclosed herein are methods of assaying the potency of an immune cell of any preceding aspect, wherein the amount of cytokine is detected using an immunoassay (such as, for example, ELISA, intracellular cytokine staining, ELISpot, flow cytometry, Luminex xMAP®, quantitative PCR (including, but not limited to qRT-PCR), and/or bead array).

[0013] In one aspect, disclosed herein are methods of assaying the potency of an immune cell of any preceding aspect wherein the immune cell is contacted with an effective amount of a stimulating agent (such as, for example, PHA, PMA/ionomycin, Con A, LPS and/or PWM) for at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 150 minutes, 3, 4, 5,6 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,

latter case, the two compounds are administered at times sufficiently close that the results observed are indistinguishable from those achieved when the compounds are administered at the same point in time. "Systemic administration" refers to the introducing or delivering to a subject an agent via a route which introduces or delivers the agent to extensive areas of the subject's body (e.g. greater than 50% of the body), for example through entrance into the circulatory or lymph systems. By contrast, "local administration" refers to the introducing or delivery to a subject an agent via a route which introduces or delivers the agent to the area or area immediately adjacent to the point of administration and does not introduce the agent systemically in a therapeutically significant amount. For example, locally administered agents are easily detectable in the local vicinity of the point of administration, but are undetectable or detectable at negligible amounts in distal parts of the subject's body. Administration includes self-administration and the administration by another.

[0050] "Treat," "treating," "treatment," and grammatical variations thereof as used herein, include the administration of a composition with the intent or purpose of partially or completely preventing, delaying, curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving, stabilizing, mitigating, and/or reducing the intensity or frequency of one or more a diseases or conditions, a symptom of a disease or condition, or an underlying cause of a disease or condition. Treatments according to the invention may be applied preventively, prophylactically, palliatively or remedially. Prophylactic treatments are administered to a subject prior to onset (e.g., before obvious signs of cancer), during early onset (e.g., upon initial signs and symptoms of cancer), or after an established development of cancer. Prophylactic administration can occur for day(s) to years prior to the manifestation of symptoms of a disease or an infection.

[0051] The methods and kits disclosed herein utilize a stimulating agent. As used herein, a stimulating agent can be any molecule, peptide, polypeptide, protein, lectin, and/or mitogen that can act as an antigen and/or immunogen to stimulate an immune cell to secrete cytokines. Stimulating agents include but are not limited to phytohemagglutinin (PHA), phorbol myristate acetate (PMA)/ionomycin, concanavalin A (Con A), lipopolysaccharide (LPS), and/or pokeweed mitogen (PWM), peanut agglutinin (PNA), wheat germ agglutinin, and ricin.

[0052] Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this pertains. The references disclosed

[0059] The assay includes the step of detecting the amount of a cytokine produced by the immune cell after stimulating the immune cells with the stimulating agent. As used herein, the term "cytokine" refers to a small protein (~5-20 kDa) that is important in cell signaling, and in particular immunomodulation that can be produced by an immune cell. Examples of cytokines include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors. The cytokines detected can include cytokines known to be produced by the immune cells being evaluated, or the detection can encompass a wider variety of cytokines, including cytokines not known to be produced by the immune cells.

[0060] In some embodiments, the cytokines being detected include cytokines known to be produced by T-cells or Natural Killer cells. In some embodiments, the cytokines include those known to be produced by T-cells. T-cells include Th1 and Th2 cells; Th1 cells predominantly produce interferon (IFN)-γ (IFN-γ), tumor necrosis factor (TNF)-α (TNF-α), and IL-2; Th2 cells produce interleukin (IL)-2 (IL-2), IL-4, IL-5, IL-6, IL-9, IL-13, and IL-22. Examples of cytokines produced by stimulated Natural Killer cells include IL-lα, IL-1β, IL- 2, IL-5, IL-8, IL-10, IL-13, IFN-γ, TNF-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), and the chemokines macrophage inflammatory protein (MIP) - 1α (MIP-1α), MIP-1β , and RANTES. Other cytokines useful to determine the potency of an immune cell include, but are not limited to B cell activating factor/ tumor necrosis factor (TNF) ligand superfamily member 13B (BAFF/TNFSF13B), cluster of differentiation (CD) 163 (CD163), CD30/TNFRSF8, Chitinase 3-like 1, gp130, IFN-α2, IL-6Rα, IL-11, IL-12(p40), IL-12(p70), IL-20, IL-26, IL-29/IFN-l1, 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 lymphopoetin (TSLP), or TNF-related weak inducer of apoptosis (TWEAK)/TNF superfamily member 12 (TWEAK/TNFSF12). Thus, in one aspect, disclosed herein are methods of assaying the potency of an immune cell (such as, for example, a T-cell, a macrophage, a NK cell, NK T cell, CAR T cell, and/or CAR NK cell), comprising contacting an immune cell with an effective amount of a stimulating agent (such as, for example, phytohemagglutinin (PHA), phorbol myristate acetate (PMA)/ionomycin, concanavalin A (Con A), lipopolysaccharide (LPS), and/or pokeweed mitogen (PWM)) and detecting the amount of one or more cytokines (such as, for example, IL-2, IL-6, IFN-γ, TNF-α, BAFF/TNFSF13B, CD163, CD30/TNFRSF8, Chitinase 3-like 1, gp130, IFN-α2, IL-6Rα, IL-8, IL-10, IL-11, IL-12(p40), IL-12(p70), IL-20, IL-22, IL-26, IL-29/IFN-l1, IL-32, IL-34, IL-35, MMP-1,