immune reaction in an individual. Lastly, there is a need to develop kits comprising biomarkers and/or other components useful to conduct the above described methods.

SUMMARY

[009] The present disclosure relates to the discovery that a cluster of differentiation 6 (CD6) is a particularly useful biomarker in identifying immunosuppressive regulatory T cells in general, as well as identifying distinct subpopulations of immunosuppressive regulatory T cells when used in conjunction with another biomarker.

[010] Accordingly, ~~aspects~~embodiments of the present specification disclose a method of identifying a population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD6 biomarker wherein detection of a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern identifies the population of immunosuppressive regulatory T-cells in the sample.

[010a] In an aspect, there is provided a method of identifying a population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of at least two different biomarkers, the at least two different biomarkers including a CD25 biomarker and a CD6 biomarker; wherein detection of a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern identifies the population of immunosuppressive regulatory T-cells in the sample.

[010b] In ~~another aspect~~one embodiment, a method of identifying a population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of at least three different biomarkers, the at least three different biomarkers including a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; wherein detection of a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern identifies the population of immunosuppressive regulatory T-cells in the sample.

[011] In another ~~aspect~~embodiment, a method of identifying a population of immunosuppressive regulatory T-cells, the method comprising contacting a sample comprising a population of T-cells with a CD6 biomarker ligand; and screening the population of T-cells to detect a level of cellular expression of a CD6 biomarker; and wherein detection of a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern identifies the population of immunosuppressive regulatory T-cells in the sample. In yet another ~~aspect~~embodiment, a method of identifying a population of immunosuppressive regulatory T-cells, the method comprising contacting a sample comprising a population of T-cells with at least two different biomarker ligands, wherein the at least two different biomarker ligands include a CD25 biomarker ligand and a CD6 biomarker ligand; and screening the population of T-cells to detect a level of cellular expression of at least two different biomarkers; wherein the at least two different biomarkers include a CD25 biomarker and a CD6 biomarker; and wherein detection of a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern identifies the population of immunosuppressive regulatory T-cells in the sample. In still another ~~aspect~~embodiment, a method of identifying a population of immunosuppressive regulatory T-cells, the method comprising contacting a sample comprising a population of T-cells with at least three different biomarker ligands, wherein the at least three different biomarker ligands include a CD4 biomarker ligand, a CD25

biomarker ligand, and a CD6 biomarker ligand; and screening the population of T-cells to detect a level of cellular expression of at least three different biomarkers; wherein the at least three different biomarkers include a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and wherein detection of a subpopulation of T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ expression pattern identifies the population of immunosuppressive regulatory T-cells in the sample.

[012] A sample may comprise T cells obtained from blood, such as, e.g., isolated from peripheral blood mononuclear cell (PBMC), lymphoid, thymus, bone marrow, or any specific tissues/organ sample of interest, including, without limitation, pancreas, eye, heart, liver, nerves, intestine, skin, muscle, cartilage, ligament, synovial fluid, and/or joints. Other biomarkers that may be used include a CD127 biomarker, a cluster of differentiation 49d (CD49d) biomarker, a cluster of differentiation 38 (CD38) biomarker, a cluster of differentiation 45RA (CD45RA) biomarker, a human leukocyte antigen serotype DR (HLA-DR) biomarker, a FoxP3 biomarker, a CTLA-4 biomarker, a GITR biomarker, a LAG-3 biomarker, a ectonucleotidase (CD39) biomarker, a member of Ikaros family zinc-finger nuclear proteins (Helios) biomarker, a orphan receptor FcRL3 (FcRL3) biomarker, a cytokine receptor 7 (CCR7 or CD197) biomarker, a cytokine receptor 4 (CCR4 or CD194) biomarker, a cytokine receptor 8 (CCR8 or CDw198), a cluster of differentiation CD62L (CD62L) biomarker, a Inducible T-cell co-stimulator (ICOS or CD278) biomarker, a cluster of differentiation 103 (CD103) biomarker, a programmed dead-1 (PD-1) biomarker, a member of the tumor necrosis factor receptor superfamily OX40 (CD134) biomarker, a glycoprotein-A repetitions predominant (GARP) biomarker, a cluster of differentiation 45RB (CD45RB) biomarker, a cluster of differentiation 45RO (CD45RO) biomarker, a cluster of differentiation 95 (CD95) biomarker, a cluster of differentiation 122 (CD122) biomarker, a cluster of differentiation 147 (CD147), a cluster of differentiation 8 (CD8) biomarker, or any combination thereof. The use of these additional biomarkers is particularly useful in identifying subpopulations of immunosuppressive regulatory T-cells. The disclosed biomarker may be antibodies or ligands and may be labeled or unlabeled. If labeled, the biomarker may be labeled with a fluorophore, a quantum dot, a phosphore, a chemiluminescent compound, a bioluminescent compound, a chromogenic compound, an isotope compound like a lanthanide, a radioisotope, an enzyme, a biotin or avidin molecule, or any other label useful for detecting a cell bound by the biomarker. The identifying step, including the samples and useful biomarkers are as disclosed herein. The identifying step may use labeled biomarkers with flow cytometer, cell sorter, magnetic particles/bead, complement lysis, cell panning or other methodologies known to one of ordinary skill in the art for isolating or enriching cells. In yet another ~~aspect~~embodiment, a disclosed method has the proviso that the biomarkers used to screen a sample does not include a FoxP3 biomarker, a CTLA-4 biomarker, or both a FoxP3 biomarker and a CTLA-4 biomarker. The methods disclosed herein may further comprise isolating or enriching the identified population of immunosuppressive regulatory T-cells. The methods disclosed herein may further comprise expanding the identified, isolated or enriched population of immunosuppressive regulatory T-cells.

[013] Other ~~aspects~~embodiments of the present specification disclose a method of obtaining a population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD6 biomarker as disclosed herein and

isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In an ~~aspect~~embodiment, a method of obtaining a population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of at least two different biomarkers, the at least two different biomarkers including a CD25 biomarker and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In another ~~aspect~~embodiment, a method of obtaining a population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of at least three different biomarkers, the at least three different biomarkers including a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells.

[014] In another ~~aspect~~embodiment, a method of obtaining a population of immunosuppressive regulatory T-cells, the method comprising contacting a sample comprising a population of T-cells with a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In yet another ~~aspect~~embodiment, a method of obtaining a population of immunosuppressive regulatory T-cells, the method comprising contacting a sample comprising a population of T-cells with at least two different biomarker ligands, wherein the at least two different biomarker ligands include a CD25 biomarker ligand and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least two different biomarkers; wherein the at least two different biomarkers include a CD25 biomarker and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In still another ~~aspect~~embodiment, a method of obtaining a population of immunosuppressive regulatory T-cells, the method comprising contacting a sample comprising a population of T-cells with at least three different biomarker ligands, wherein the at least three different biomarker ligands include a CD4 biomarker ligand, a CD25 biomarker ligand, and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least three different biomarkers; wherein the at least three different biomarkers include a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells.

[015] The identifying step, including the samples and useful biomarkers are as disclosed herein. The isolating step may use labeled biomarkers with flow cytometer, cell sorter, magnetic particles/bead, or other methodologies known to one of ordinary skill in the art for isolating cells. The identifying and isolating can proceed in any order or simultaneously by the use of multiple, distinguishable biomarkers used to detect the common determinants. In yet another ~~aspect~~embodiment, a disclosed method has the proviso that the biomarkers used to screen and/or isolate a sample does not include a FoxP3 biomarker, a CTLA-4 biomarker, or both a FoxP3 biomarker and a CTLA-4 biomarker. The methods disclosed herein may

further comprise enriching or expanding the isolated subpopulation of immunosuppressive regulatory T-cells.

[016] Yet other ~~aspects~~embodiments of the present specification disclose a method of obtaining an expanded population of immunosuppressive regulatory T-cells, the method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD6 biomarker as disclosed herein, isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern as disclosed herein, and contacting the subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern with a stimulatory composition, thereby obtaining the expanded population of immunosuppressive regulatory T-cells. In an ~~aspect~~embodiment, a method of obtaining an expanded population of immunosuppressive regulatory T-cells, the method comprising the steps of screening a sample comprising a population of T-cells to detect a level of cellular expression of at least two different biomarkers, the at least two different biomarkers including a CD25 biomarker and a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD25^+$ $CD6^{low/-}$ expression pattern; and contacting the subpopulation of T-cells comprising a $CD25^+$ $CD6^{low/-}$ expression pattern with a stimulatory composition, thereby obtaining the expanded population of immunosuppressive regulatory T-cells. In another ~~aspect~~embodiment, a method of obtaining an expanded population of immunosuppressive regulatory T-cells, the method comprising the steps of screening a sample comprising a population of T-cells to detect a level of cellular expression of at least three different biomarkers, the at least three different biomarkers including a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ expression pattern; and contacting the subpopulation of T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ expression pattern with a stimulatory composition, thereby obtaining the expanded population of immunosuppressive regulatory T-cells.

[017] In another ~~aspect~~embodiment, a method of obtaining an expanded population of immunosuppressive regulatory T-cells, the method comprising the steps of contacting a sample comprising a population of T-cells with a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern; and contacting the subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern with a stimulatory composition, thereby obtaining the expanded population of immunosuppressive regulatory T-cells. In yet another ~~aspect~~embodiment, a method of obtaining an expanded population of immunosuppressive regulatory T-cells, the method comprising the steps of contacting a sample comprising a population of T-cells with at least two different biomarker ligands, wherein the at least two different biomarker ligands include a CD25 biomarker ligand and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least two different biomarkers; wherein the at least two different biomarkers include a CD25 biomarker and a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD25^+$ $CD6^{low/-}$ expression pattern; and contacting the subpopulation of T-cells comprising a $CD25^+$ $CD6^{low/-}$ expression pattern with a stimulatory composition, thereby obtaining the expanded population of immunosuppressive regulatory T-cells. In still another ~~aspect~~embodiment, a method of obtaining an expanded population of immunosuppressive regulatory T-cells, the method comprising the steps of contacting a sample comprising a population of T-cells with at least three different biomarker ligands,

wherein the at least three different biomarker ligands include a CD4 biomarker ligand, a CD25 biomarker ligand, and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least three different biomarkers; wherein the at least three different biomarkers include a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ expression pattern; and contacting the subpopulation of T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ expression pattern with a stimulatory composition, thereby obtaining the expanded population of immunosuppressive regulatory T-cells.

[018] The identifying step, including the samples and useful biomarkers as well as the isolating step, including labeled biomarkers and methodologies are as disclosed herein. A stimulatory composition comprises a TCR/CD3 activator that is antigen-specific and may further comprise one or more additional agents including a co-stimulatory agent, a second regulatory T cell stimulatory agent, or a T cell survival or growth agent. Alternatively, the subpopulation T-cells with a desired expression pattern can first be enriched or expanded and then isolated as disclosed herein.

[019] Still other ~~aspects~~embodiments of the present specification disclose a method of modulating an immune reaction in an individual, the method comprising obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells; screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern; and administering the subpopulation T-cells with a $CD6^{low/-}$ expression pattern into the individual, thereby treating an immune-based disorder in the individual.

[019a] In one aspect, there is provided the use of a subpopulation T-cells with a $CD25^+CD6^{low/-}$ expression pattern in the manufacture of a medicament for treating an immune-based disorder in an individual, wherein the subpopulation of T-cells with a $CD25^+CD6^{low/-}$ expression pattern is obtained by:

obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells;

screening a sample comprising a population of T-cells to detect a level of cellular expression of at least two different biomarkers, the at least two different biomarkers including a CD25 biomarker and a CD6 biomarker; and

isolating a subpopulation of T-cells comprising a $CD25^+CD6^{low/-}$ expression pattern;

wherein the subpopulation T-cells with a $CD25^+CD6^{low/-}$ expression pattern is to be administered into the individual, thereby treating the immune reaction in the individual, and wherein the immune-based disorder is selected from a group consisting of an autoimmune condition, autoimmune disorder, autoimmune disease, a transplant rejection, a graft vs. host disease, a cancer, immune-based inflammatory diseases, allergies, asthma, persistent and progressive immune reactions to infectious non self-antigens that persist within mammals and humans and combinations of the immune-based disorders, and wherein the antigen is selected from a group consisting of bacterial, viral, fungal, or parasitic organisms.

[019b] In an ~~aspect~~embodiment, a method of modulating an immune reaction in an individual, the method comprising obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells; screening a sample comprising a population of T-cells to detect a level of cellular expression of at least two different biomarkers, the at least two different biomarkers including a CD25 biomarker and a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern; and administering the subpopulation T-cells with a $CD25^{+}$ $CD6^{low/-}$ expression pattern into the individual, thereby treating an immune-based disorder in the individual.

[019c] In another ~~aspect~~embodiment, a method of modulating an immune reaction in an individual, the method comprising obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells; screening a sample comprising a population of T-cells to detect a level of cellular expression of at least three different biomarkers, the at least three different biomarkers including a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; isolating a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern; and administering the subpopulation T-cells with a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern into the individual, thereby treating an immune-based disorder in the individual.

[020] In another ~~aspect~~embodiment, a method of modulating an immune reaction in an individual, the method comprising obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells; contacting a sample comprising a population of T-cells with a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern; and administering the

subpopulation T-cells with a $CD6^{low/-}$ expression pattern into the individual, thereby treating an immune-based disorder in the individual. In yet another ~~aspect~~embodiment, a method of modulating an immune reaction in an individual, the method comprising obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells; contacting a sample comprising a population of T-cells with at least two different biomarker ligands, wherein the at least two different biomarker ligands include a CD25 biomarker ligand and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least two different biomarkers; wherein the at least two different biomarkers include a CD25 biomarker and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern; and administering the subpopulation T-cells with a $CD25^{+}$ $CD6^{low/-}$ expression pattern into the individual, thereby treating an immune-based disorder in the individual. In still another ~~aspect~~embodiment, a method of modulating an immune reaction in an individual, the method comprising obtaining a biological sample, the biological sample comprising a population of individual-compatible T-cells; contacting a sample comprising a population of T-cells with at least three different biomarker ligands, wherein the at least three different biomarker ligands include a CD4 biomarker ligand, a CD25 biomarker ligand, and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least three different biomarkers; wherein the at least three different biomarkers include a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern; and administering the subpopulation T-cells with a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern into the individual, thereby treating an immune-based disorder in the individual.

[021] The identifying step, including the samples and useful biomarkers as well as the isolating step, including labeled biomarkers and methodologies are as disclosed herein. The disclosed methods may further comprise enriching or expanding the subpopulation T-cells with a desired expression pattern as disclosed herein prior to administering the subpopulation T-cells into the individual. Alternatively, the population of individual-compatible T-cells in the biological sample can be enriched or expanded before screening the sample as disclosed herein and/or before isolating the subpopulation T-cells with a desired expression pattern as disclosed herein.

[022] Other ~~aspects~~embodiments of the present specification disclose a kit comprising components useful in performing any of the methods disclosed herein. In an ~~aspect~~embodiment, a kit for identifying and/or isolating or enriching a population of immunosuppressive regulatory T-cells comprises a CD6 biomarker ligand. In one~~another~~ aspect, there is provided a kit for identifying, isolating and/or enriching a population of immunosuppressive regulatory T-cells, the kit comprising ~~comprises~~ at least two different biomarker ligands, ~~wherein~~ the at least two different biomarker ligands including~~include~~ a CD25 biomarker ligand and a CD6 biomarker ligand. In yet another ~~aspect~~embodiment, a kit for identifying, isolating and/or enriching a population of immunosuppressive regulatory T-cells comprises at least three different biomarker ligands, wherein the at least three different biomarker ligands include a CD4 biomarker ligand, a CD25 biomarker ligand, and a CD6 biomarker ligand. Such disclosed kits may further include a CD127 biomarker ligand, a CD49d biomarker ligand, a CD38 biomarker ligand, a CD45RA biomarker ligand, a HLA-DR biomarker ligand, a FoxP3 biomarker ligand, a CTLA-4 biomarker ligand, a GITR biomarker ligand, a LAG-3 biomarker ligand, a CD39 biomarker ligand, a Helios biomarker

ligand, a FcRL3 biomarker ligand, a CCR7 biomarker ligand, a CCR4 biomarker ligand, a CCR8 biomarker ligand, a CD62L biomarker ligand, a ICOS biomarker ligand, a CD103 biomarker ligand, a PD-1 biomarker ligand, a CD134 biomarker ligand, a GARP biomarker ligand, a CD45RB biomarker ligand, a CD45RO biomarker ligand, a CD95 biomarker ligand, a CD122 biomarker ligand, a CD147 biomarker ligand, a CD8 biomarker ligand, or any combination thereof. The disclosed biomarker ligands may be antibodies or ligands and may be labeled or unlabeled. If labeled, the biomarker ligand may be labeled with a fluorophore, a quantum dot, a phosphore, a chemiluminescent compound, a bioluminescent compound, a chromogenic compound, an isotope compound like a lanthanide, a radioisotope, an enzyme, a biotin or avidin molecule, or any other label useful for detecting a cell bound by the biomarker. If unlabeled, the kit may further include a label and other reagents useful for labeling a biomarker ligand. In yet another ~~aspect~~embodiment, a kit for expanding a population of immunosuppressive regulatory T-cells comprises a stimulatory composition as disclosed herein. Such a kit may further comprise a CD6 biomarker ligand or at least three different biomarker ligands as disclosed herein. A disclosed kit may further comprise positive and/or negative controls. Additionally, a disclosed kit may further comprise instructions for identifying, isolating, enriching, and/or expanding a population of immunosuppressive regulatory T-cells by use of the kit's contents.

[023] Yet other ~~aspects~~embodiments of the present specification disclose a composition comprising a population of immunosuppressive regulatory T-cells obtained by a method of obtaining a population of immunosuppressive regulatory T-cells as disclosed herein. In an ~~aspect~~embodiment, a population of immunosuppressive regulatory T-cells is obtained by a method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD6 biomarker as disclosed herein and isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In another ~~aspect~~embodiment, a population of immunosuppressive regulatory T-cells is obtained by a method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of the at least two different biomarkers, the at least two different biomarkers including a CD25 biomarker and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In yet another ~~aspect~~embodiment, a population of immunosuppressive regulatory T-cells is obtained by a method comprising screening a sample comprising a population of T-cells to detect a level of cellular expression of at least three different biomarkers, the at least three different biomarkers including a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells.

[023a] In one aspect, there is provided a composition comprising a population of immunosuppressive regulatory T-cells, the population of immunosuppressive regulatory T-cells obtained by a method comprising the steps:

screening a sample comprising a population of T-cells to detect a level of cellular expression of the at least three different biomarkers, the at least three different biomarkers including a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and

isolating a subpopulation of T-cells comprising a $CD4^{+}CD25^{+}CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells.

[024] In another ~~aspect~~embodiment, a population of immunosuppressive regulatory T-cells is obtained by a method comprising contacting a sample comprising a population of T-cells with a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In yet another ~~aspect~~embodiment, a population of immunosuppressive regulatory T-cells is obtained by a method comprising contacting a sample

comprising a population of T-cells with at least two different biomarker ligands, wherein the at least two different biomarker ligands include a CD25 biomarker ligand and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least two different biomarkers; wherein the at least two different biomarkers include a CD25 biomarker and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells. In still another ~~aspect~~embodiment, a population of immunosuppressive regulatory T-cells is obtained by a method comprising contacting a sample comprising a population of T-cells with at least three different biomarker ligands, wherein the at least three different biomarker ligands include a CD4 biomarker ligand, a CD25 biomarker ligand, and a CD6 biomarker ligand; screening the population of T-cells to detect a level of cellular expression of at least three different biomarkers; wherein the at least three different biomarkers include a CD4 biomarker, a CD25 biomarker, and a CD6 biomarker; and isolating a subpopulation of T-cells comprising a $CD4^{+}$ $CD25^{+}$ $CD6^{low/-}$ expression pattern, thereby obtaining the population of immunosuppressive regulatory T-cells.

[025] The identifying step, including the samples and useful biomarkers are as disclosed herein. The isolating step may use labeled biomarkers with flow cytometer, cell sorter, magnetic particles/bead, complement lysis, cell panning or other methodologies known to one of ordinary skill in the art for isolating cells. The identifying and isolating can proceed in any order or simultaneously by the use of multiple, distinguishable biomarkers used to detect the common determinants. The methods disclosed herein may further comprise enriching or expanding the identified population of immunosuppressive regulatory T-cells.

[026] Still other ~~aspects~~embodiments of the present specification disclose a use of a composition comprising a population of immunosuppressive regulatory T-cells as disclosed herein to treat an immune response. An immune response may be an autoimmune response.

[026a] In one aspect, there is provided the use of an α-CD6 antibody in the manufacture of a medicament for enriching immunosuppressive regulatory T-cells in an individual, wherein the administration renders inoperable T-cells comprising a $CD6^{hi/+}$ expression pattern, but not T-cells comprising a $CD6^{low/-}$ expression pattern, thereby enriching for a population of immunosuppressive regulatory T-cells in the individual, and wherein the individual having an immune-based disorder, the immune-based disorder is selected from a group consisting of an autoimmune condition, autoimmune disorder, autoimmune disease, a transplant rejection, a graft vs. host disease, a cancer, immune-based inflammatory diseases, allergies, asthma, persistent and progressive immune reactions to infectious non self-antigens that persist within mammals and humans and combinations of the immune-based disorders, and wherein the antigen is selected from a group consisting of bacterial, viral, fungal, or parasitic organisms.

[027] Other ~~aspects~~embodiments of the present specification disclose a method of enriching immunosuppressive regulatory T-cells in an individual, the method comprising administering an α-CD6 antibody to the individual, wherein the administration renders inoperable T-cells comprising a $CD6^{hi/+}$ expression pattern, but not T-cells comprising a $CD6^{low/-}$ expression pattern, thereby enriching for a population of immunosuppressive regulatory T-cells in the individual.

[027a] In one aspect, there is provided a method of identifying two or more distinct immunosuppressive regulatory T-cell populations, the method comprising the steps of

screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD4 biomarker, a CD25 biomarker, a CD6 biomarker, and one additional biomarker, the one additional biomarker being a FoxP3 biomarker, a CTLA-4 biomarker, a CD45RA biomarker, or a HLA-Dr biomarker;

wherein detection of two distinct populations of immunosuppressive regulatory T-cells is based upon i) T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ $FoxP3^+$ expression pattern and $CD4^+$ $CD25^+$ $CD6^{low/-}$ $FoxP3^{low/-}$ expression pattern, ii) T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ $CTLA\text{-}4^+$ expression pattern and $CD4^+$ $CD25^+$ $CD6^{low/-}$ $CTLA\text{-}4^{low/-}$ expression pattern, iii) T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ $CD45RA^+$ expression pattern and $CD4^+$ $CD25^+$ $CD6^{low/-}$ $CD45RA^{low/-}$ expression pattern, or iv) T-cells comprising a $CD4^+$ $CD25^+$ $CD6^{low/-}$ $HLA\text{-}Dr^+$ expression pattern and $CD4^+$ $CD25^+$ $CD6^{low/-}$ $HLA\text{-}Dr^{low/-}$ expression pattern.

[028] In one aspect, there is provided~~Other aspects of the present specification disclose~~ a method of identifying four distinct maturation subsets of immunosuppressive regulatory T-cell populations,~~In an aspect,~~ the method comprising~~may comprises~~ the steps of screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD4 biomarker, a CD25 biomarker, a CD6 biomarker, and one additional biomarker, the one additional biomarker being a FoxP3 biomarker, a CD45RA biomarker, a CCR4 biomarker, a CD39 biomarker, or a HLA-Dr biomarker; wherein detection of the four distinct maturation subsets of immunosuppressive regulatory T-cells is based upon an expression pattern comprising a i) $CD4^+CD25^+CD6^{low/-}CD45RA^+$ expression pattern in conjunction with a $CCR4^{low}$ expression pattern, a $CD39^{low}$ expression pattern, or a $HLA\text{-}Dr^{low}$

expression pattern; ii) a $CD4^+CD25^+CD6^{low/-}CD45RA^+$ expression pattern in conjunction with a $CCR4^-$ expression pattern, a $CD39^-$ expression pattern, or a $HLA\text{-}Dr^-$ expression pattern; iii) a $CD4^+CD25^{hi/+}CD6^{low/-}$ expression pattern in conjunction with a $CCR4^{hi/+}$ expression pattern, a $CD45RA^-$ expression pattern, a $CD39^{hi/+}$ expression pattern, or a $HLA\text{-}Dr^{low/-}$ expression pattern; and iv) a $CD4^+CD25^+CD6^+$ expression pattern in conjunction with $CR4^{hi/+}$ expression pattern, a $CD45RA^-$ expression pattern, a $CD39^{low/-}$ expression pattern, or a $HLA\text{-}Dr^{low/-}$ expression pattern.

[028a] In one aspect, there is provided a method of identifying four distinct maturation subsets of immunosuppressive regulatory T-cell populations, the method comprising the steps of

screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD4 biomarker, a CD25 biomarker, a CD127 biomarker, and one additional biomarker, the one additional biomarker being a FoxP3 biomarker, a CD45RA biomarker, a CCR4 biomarker, a CD39 biomarker, or a HLA-Dr biomarker;

wherein detection of the four distinct maturation subsets of immunosuppressive regulatory T-cells is based upon an expression pattern comprising i) a $CD4^+CD25^+CD127^{low/-}CD45RA^+$ expression pattern in conjunction with a $CCR4^{low}$ expression pattern, a $CD39^{low}$ expression pattern, or a $HLA\text{-}Dr^{low}$ expression pattern; ii) a $CD4^+CD25^+CD127^{low/-}CD45RA^+$ expression pattern in conjunction with a $CCR4^-$ expression pattern, a $CD39^-$ expression pattern, or a $HLA\text{-}Dr^-$ expression pattern; iii) a $CD4^+CD25^{hi/+}CD127^{low/-}$ expression pattern in conjunction with a $CCR4^{hi/+}$ expression pattern, a $CD45RA^-$ expression pattern, a $CD39^{hi/+}$ expression pattern, or a $HLA\text{-}Dr^{low/-}$ expression pattern; and iv) a $CD4^+CD25^+CD127^+$ expression pattern in conjunction with $CR4^{hi/+}$ expression pattern, a $CD45RA^-$ expression pattern, a $CD39^{low/-}$ expression pattern, or a $HLA\text{-}Dr^{low/-}$ expression pattern.

[028b] In another ~~aspect~~embodiment, the method may comprises the step of screening a sample comprising a population of T-cells to detect a level of cellular expression of a CD4 biomarker, a CD25 biomarker, a CD127 biomarker, and one additional biomarker, the one additional biomarker being a FoxP3 biomarker, a CD45RA biomarker, a CCR4 biomarker, a CD39 biomarker, or a HLA-Dr biomarker; wherein detection of the four distinct maturation subsets of immunosuppressive regulatory T-cells is based upon a i) $CD4^+CD25^+CD127^{low/-}CD45RA^+$ expression pattern in conjunction with a $CCR4^{low}$ expression pattern, a $CD39^{low}$ expression pattern, or a $HLA\text{-}Dr^{low}$ expression pattern; ii) a $CD4^+CD25^+CD127^{low/-}CD45RA^+$ expression pattern in conjunction with a $CCR4^-$ expression pattern, a $CD39^-$ expression pattern, or a $HLA\text{-}Dr^-$ expression pattern; iii) a $CD4^+CD25^{hi/+}CD127^{low/-}$ expression pattern in conjunction with a $CCR4^{hi/+}$ expression pattern, a $CD45RA^-$ expression pattern, a $CD39^{hi/+}$ expression pattern, or a $HLA\text{-}Dr^{low/-}$ expression pattern; and iv) a $CD4^+CD25^+CD127^+$ expression pattern in conjunction with $CR4^{hi/+}$ expression pattern, a $CD45RA^-$ expression pattern, a $CD39^{low/-}$ expression pattern, or a $HLA\text{-}Dr^{low/-}$ expression pattern. The four distinct maturation subsets of immunosuppressive regulatory T-cell populations, include, without limitation, a naïve or resting immunosuppressive regulatory T-cell population subset, an immature or memory immunosuppressive regulatory T-cell population subset, a mature or effector immunosuppressive regulatory T-cell population subset, and a terminal differentiated immunosuppressive regulatory T-cell population subset.

BRIEF DESCRIPTION OF THE DRAWINGS

[029] **FIG. 1A** shows dotplots analyzing $CD4^+$ lymphocytes for CD4/FOXP3 and CD25/FOXP3 expression; and **FIG. 1B** shows an overlay plot of low or negative expression of CD6 on $CD4^+FOXP3^+$ and $CD25^+FOXP3^+$ cell populations as compare with $CD4^+FOXP3^-$ and $CD4^+CD25^+FOXP3^-$ cell population respectively.

[030] **FIG. 2A** shows dotplots analyzing $CD4^+$ lymphocytes; and **FIG 2B** shows dotplots analyzing $CD4^+$ lymphocytes for CD6/CD25 expression and the expression of FOXP3 on three different $CD4^+$ cell subsets: $CD25^+CD6^{low/-}$, $CD25^-CD6^{low/-}$ and $CD25^-CD6^+$.

[031] **FIG. 3** shows dotplots analyzing lymphocytes for CD6/CD25 expression and the expression of FOXP3 on three different lymphocyte subsets: $CD25^+CD6^{low/-}$, $CD25^-CD6^{low/-}$ and $CD25^-CD6^+$.

[032] **FIG. 4A** shows dotplots analyzing PBMC for FOXP3 expression in $CD6^{low/-}$ and $CD6^+$ cell populations; and **FIG. 4B** shows a graph of % of $FOXP3^+$ cell expression in total PBMC, PBMC $CD6^{low/-}$ and PBMC $CD6^+$ cell populations.