

improvements, in comparison to assays known in the art. Processes for manufacturing, methods of administration, and pharmaceutical compositions using potency assays are also described. These processes and methods may additionally be used with the administration of TILs in combination with CTLA-4 and PD-1 inhibitors and/or PD-L1 inhibitors as described herein.

SUMMARY

[0004] Provided herein are methods for assessing the potency and/or functionality of expanded TILs and other polyclonal T cell products, including marrow infiltrating lymphocytes (MILs) and peripheral blood lymphocytes (PBLs), which can then be employed in the treatment of cancer by administering TILs, MILs, PBLs, or other polyclonal T cell products assessed by these methods.

[0005] In one aspect, the present disclosure provides a method of determining the potency of a TIL, the method comprising the steps of: a. performing a co-culture of a target cell with a TIL cell for a first period; b. obtaining a harvest or extracting a supernatant from the co-culture; and c. assessing (1) the harvest for expression of one or more markers on the TIL cell or (2) the supernatant for one or more analytes secreted from the TIL cell to obtain one or more observed values to determine the potency for the TIL. In one example, the present disclosure provides for a method of determining the potency of a T cell product, the method comprising the steps of:

- a. performing a co-culture of a target cell with a T cell product cell for a first period;
- b. obtaining a harvest from the co-culture; and
- c. assessing the harvest for (1) expression of one or more markers on the T cell product cell or (2) one or more analytes secreted from the T cell product cell to obtain one or more observed values to determine the potency for the T cell product.

[0006] In some examples, the method comprises the additional steps of:

- d. performing a second co-culture of a negative control comprising (i) a negative control cell or (ii) a human leukocyte antigen (HLA) blocking antibody and the target cell with the T cell product cell for a second period;
- e. obtaining a second harvest from the second co-culture;
- f. assessing the second harvest for (1) the expression of the one or more markers on the T cell product cell or (2) the one or more analytes secreted from the T cell product cell to obtain one or more control values; and
- g. comparing the one or more observed values from step c with the one or more control values from step f, where each observed value is compared to its corresponding control value, to determine the potency of the T cell product.

- [0007]** In some examples, the T cell product is selected from the group consisting of a tumor-infiltrating lymphocyte (TIL) product, a marrow-infiltrating lymphocyte (MIL) product, or a peripheral blood lymphocyte (PBL) product.
- [0008]** In some examples, the T cell product is a TIL product from a human, and wherein the TIL product is obtained by resection of a tumor or fragmentation or digestion of a tumor and manufactured by a TIL expansion process comprising a rapid expansion protocol step.
- [0009]** In some examples, the method comprises the step of releasing the T cell product for use in the treatment of a human patient.
- [0010]** In some example, the target cell is an irradiated Raji cell or a derivative, variant, modification, or progeny thereof.
- [0011]** In some examples, the negative control cell lacks MHC or HLA Class I and MHC or HLA Class II expression.
- [0012]** In some examples, the negative control cell is an irradiated K562 cell or a derivative, variant, modification, or progeny thereof.
- [0013]** In some examples, the HLA blocking antibody comprises an HLA-I blocking antibody, an HLA-II blocking antibody, or a combination thereof.
- [0014]** In some examples, the ratio between the number of TIL product cells to the number of target cells is between 5:1 and 1:5.
- [0015]** In some examples, the ratio between the number of TIL product cells to the number of negative control cells is between 5:1 and 1:5.
- [0016]** In some examples, the ratio between the number of TIL product cells to the number of target cells is between 3:1 and 1:3.
- [0017]** In some examples, the ratio between the number of TIL product cells to the number of negative control cells is between 3:1 and 1:3.
- [0018]** In some examples, the ratio between the number of TIL product cells to the number of target cells is between 1:2 and 1:4.
- [0019]** In some examples, the ratio between the number of TIL product cells to the number of negative control cells is between 1:2 and 1:4.

[0020] In some examples, the ratio between the number of TIL product cells to the number of target cells is between 1:25 and 1:35.

[0021] In some examples, the ratio between the number of TIL product cells to the number of negative control cells is between 1:25 and 1:35.

[0022] In some examples, the ratio between the number of TIL product cells to the number of target cells is about 1:2.

[0023] In some examples, the ratio between the number of TIL product cells to the number of negative control cells is about 1:2.

[0024] In some examples, the ratio between the number of TIL product cells to the number of target cells is about 1:3.

[0025] In some examples, the ratio between the number of TIL product cells to the number of negative control cells is about 1:3.

[0026] In some examples, the ratio between the number of TIL product cells to the number of target cells is about 1:4.

[0027] In some examples, the ratio between the number of TIL product cells to the number of negative control cells is about 1:4.

[0028] In some examples, the co-culture is performed after thawing a cryopreserved TIL product, MIL product, or PBL product, and allowing the thawed TIL product, MIL product, or PBL product to recover under incubation for a period selected from the group consisting of 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, and 72 hours.

[0029] In some examples, the first period is from about 6 hours to about 48 hours.

[0030] In some examples, the second period is from about 6 hours to about 48 hours.

[0031] In some examples, the first period is selected from the group consisting of about 12 hours, about 18 hours, and about 24 hours.

[0032] In some examples, the second period is selected from the group consisting of about 12 hours, about 18 hours, and about 24 hours.

[0033] In some examples, the first period and the second period are the same duration.

[0034] In some examples, the one or more markers on the T cell product are selected from the group consisting of CD25, CD69, CD134, CD137, CD150, KLRG1, or combinations thereof.

[0035] In some examples, the one or more analytes secreted from the T cell product is selected from the group consisting of IFN- α , IFN- β , IFN- γ , granzyme B, perforin, TNF- α , IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-14, IL-16, IL-17, IL-18, IL-22, IL-25, IL-26, MIP-1 β , and combinations thereof.

[0036] In some examples, the one or more analytes secreted from the TIL product is selected from the group consisting of IFN- α , IFN- β , IFN- γ , granzyme B, perforin, TNF- α , IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-14, IL-16, IL-17, IL-18, IL-22, IL-25, IL-26, MIP-1 β , and combinations thereof, wherein the quantity of the observed value is normalized to the quantity of the control value for each of the one or more analytes, and wherein the increase in observed value over the control value for each of the one or more analytes is selected from the group consisting of at least 1-fold, at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold, at least 4-fold, at least 4.5-fold, and at least 5-fold.

[0037] In some examples, the TIL product is manufactured from a tumor obtained by surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a human patient.

[0038] In another example, the present disclosure provides a method of treating a cancer in a patient in need thereof with a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

- (a) obtaining and/or receiving a first population of TILs from a tumor resected from the patient by surgical resection, needle biopsy, core biopsy, small biopsy, or other means by processing a tumor sample obtained from the patient into (i) multiple tumor fragments or (ii) a tumor digest;
- (b) adding the first population of TILs into a closed system;
- (c) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-14 days to obtain the second population of TILs, and wherein the transition from step

- (b) to step (c) occurs without opening the system;
- (d) performing a second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and antigen presenting cells (APCs), to produce a third population of TILs, wherein the second expansion is performed for about 7-14 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (c) to step (d) occurs without opening the system;
- (e) harvesting the therapeutic population of TILs obtained from step (d), wherein the transition from step (d) to step (e) occurs without opening the system;
- (f) determining the potency of the therapeutic population of TILs by:
 - i. performing a co-culture of a target cell with a portion of the therapeutic population of TILs for a first period;
 - ii. obtaining a harvest from the co-culture;
 - iii. assessing the harvest for (1) expression of one or more markers on the portion of the therapeutic population of TILs or (2) one or more analytes secreted from the portion of the therapeutic population of TILs to obtain one or more observed values to determine the potency for the therapeutic population of TILs;
 - iv. performing a second co-culture of a negative control cell or (i) a negative control cell or (ii) a human leukocyte antigen blocking antibody with a second portion of the therapeutic population of TILs for a second period;
 - v. obtaining a second harvest from the second co-culture;
 - vi. assessing the second harvest for (1) the expression of the one or more markers on the second portion of the therapeutic population of TILs or (2) the one or more analytes secreted from the second portion of the therapeutic population of TILs to obtain one or more control values; and
 - vii. comparing the one or more observed values with the one or more control values, where each observed value is compared to its corresponding control value, to determine the potency of the therapeutic population of

TILs;

- (g) transferring the therapeutic population of TILs from step (e) to an infusion bag, wherein the transfer from step (e) to (g) occurs without opening the system;
- (h) optionally cryopreserving the infusion bag comprising the therapeutic population of TILs from step (g) using a cryopreservation process; and
- (i) if the therapeutic population of TILs is determined to be potent, administering a therapeutically effective dosage of the therapeutic population of TILs from the infusion bag in step (g) or (h) to the patient.

[0039] In some examples, examining the potency and/or functionality of the TILs harvested occurs after cryopreservation, or optionally before and after a cryopreservation step.

[0040] In some examples, the patient has a tumor that is unresectable, metastatic, resistant, or refractory to a CTLA-4 inhibitor, PD-1 inhibitor, or a PD-L1 inhibitor, and optionally wherein the patient has been previously treated with a CTLA-4 inhibitor, a PD-1 inhibitor, or a PD-L1 inhibitor.

[0041] In some examples, the second population of TILs in step (c) is at least 50-fold greater in number than the first population of TILs.

[0042] In some examples, the first expansion is performed over a period of about 10 to about 12 days.

[0043] In some examples, the second expansion is performed over a period of about 10 to about 12 days.

[0044] In some examples, the first expansion is performed over a period of about 11 days.

[0045] In some examples, the second expansion is performed over a period of about 11 days.

[0046] In some examples, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL in the cell culture medium in the first expansion.

[0047] In some examples, in the second expansion step, the IL-2 is present at an initial concentration of between 1000 IU/mL and 6000 IU/mL and the OKT-3 antibody is present at an initial concentration of about 30 ng/mL.

[0048] In some examples, the method further comprises the step of treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the TILs to the patient.

[0049] In some examples, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for five days.

[0050] In some examples, the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m²/day and fludarabine at a dose of 25 mg/m²/day for two days followed by administration of fludarabine at a dose of 25 mg/m²/day for three days.

[0051] In some examples, the method further comprises the step of treating the patient with an IL-2 regimen starting on the day after the administration of the therapeutic population of TILs to the patient.

[0052] In some examples, the method further comprises the step of treating the patient with an IL-2 regimen starting on the same day as administration of the therapeutic population of TILs to the patient.

[0053] In some examples, the IL-2 regimen is administered about 3 to about 24 hours after completion of the administration of the therapeutic population of TILs to the patient.

[0054] In some examples, the IL-2 regimen is a high-dose IL-2 regimen comprising 600,000 or 720,000 IU/kg of aldesleukin, or a biosimilar or variant thereof, administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

[0055] In some examples, processing a tumor sample obtained from the patient into a tumor digest in step (a) further comprises incubating the tumor sample in an enzymatic media.

[0056] In some examples, processing a tumor sample obtained from the patient into a tumor digest in step (a) further comprises disrupting the tumor sample mechanically so as to dissociate the tumor sample.

[0057] In some examples, processing a tumor sample obtained from the patient into a tumor digest in step (a) further comprises purifying the disassociated tumor sample using a density gradient separation.

[0058] In some examples, the enzymatic media comprises DNase.

- [0059]** In some examples, the enzymatic media comprises about 30 units/mL of DNase.
- [0060]** In some examples, the enzymatic media comprises collagenase.
- [0061]** In some examples, the enzymatic media comprises about 1.0 mg/mL of collagenase.
- [0062]** In some examples, the cancer is selected from the group consisting of melanoma, ovarian cancer, pancreatic cancer, endometrial cancer, thyroid cancer, cervical cancer, non-small-cell lung cancer, small-cell lung cancer, bladder cancer, breast cancer, head and neck cancer, glioblastoma, gastrointestinal cancer, renal cancer, sarcoma, and renal cell carcinoma.
- [0063]** In some examples, the method comprises the step of administering a PD-1 inhibitor, PD-L1 inhibitor, or CTLA-4 inhibitor to the patient.
- [0064]** In another example, the present disclosure provides a method of determining the potency of a T cell product, the method comprising the steps of:
- a. performing at least three co-cultures of target cells with T cell product cells at different target cell concentrations;
 - b. performing at least three co-cultures of target cells with T cell reference standard cells at different target cell concentrations;
 - c. extracting supernatants from each of the co-cultures; and
 - d. assessing the supernatants for a cytokine secreted from the T cell product cells and T cell reference standard cells to obtain dose-concentrations to determine the potency of the T cell product;
- wherein the target cells are monocyte cells.
- [0065]** In some examples, the T cell product is a tumor infiltrating lymphocyte (TIL) product, a marrow infiltrating lymphocyte (MIL) product, or a peripheral blood lymphocyte (PBL) product.
- [0066]** In some examples, the monocyte cells are U937 or Thp1 cells, or a derivative, variant, modification, or progeny thereof, and wherein the cytokine is interferon- γ .
- [0067]** In some examples, the co-cultures are performed for a time period selected from the group consisting of about 6 hours, about 12 hours, about 18 hours, about 24 hours, about 30 hours, about 36 hours, about 42 hours, and about 48 hours.

[0068] In some examples, the T cell product is a TIL product, and wherein four target cell dose-concentrations of about 4×10^5 , about 2×10^5 , about 1×10^5 , and about 0.5×10^5 target cells per well and a single TIL cell concentration of about 1.5×10^6 TIL per well are used.

[0069] In some examples, at least four co-cultures of target cells with T cell product cells and at least four co-cultures of target cells with T cell reference standard cells are used, parallel line analysis is performed, and one outlier target cell dose-concentration is discarded.

[0070] In some examples, the method is a component of a potency assay matrix.

[0071] In some examples, the potency assay matrix comprises one or more assays selected from the group consisting of a bead- or plate-based assay using CD3, CD28, and/or CD137 stimulation and reporting interferon- γ , granzyme B, or tumor necrosis factor- α , an assay for total viable cells, an assay for percentage viable cells, an assay for CD4⁺ cell content, an assay for CD8⁺ cell content, an assay for T_{EM} cell content, an assay for T_{CM} cell content, an assay for LAG3⁺ cell content, and an assay for KLRG1⁺ cell content, an assay for CD101⁺ cell content, an assay for CD69⁺ cell content, an assay for T_{SCM} cell content, an assay for T_{EMRA} cell content, an assay for T_{reg} cell content, an assay for PD-1⁺ cell content, an assay for TIM3⁺ cell content, an assay for CD25⁺ cell content, an assay for CD27⁺ cell content, an assay for CD28⁺ cell content, an assay for CD56⁺ cell content, an assay for CTLA-4⁺ cell content, an assay for TIGIT⁺ cell content, and an assay for CD57⁺ cell content.

[0072] In another example, the present disclosure provides a method for treating a subject with a cancer, the method comprising administering an expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) adding a tumor digest or tumor fragments into a closed system, wherein the tumor digest or tumor fragments comprise a first population of TILs and are obtained from a tumor that was resected from the subject;
- (b) performing a first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2 to produce a second population of TILs, wherein the first expansion is performed in a closed container providing a first gas-permeable surface area, wherein the first expansion is performed for about 3-11 days to obtain the second population of TILs, and wherein the transition from step (b) to step (c) occurs without opening the system;
- (c) performing a second expansion by supplementing additional cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs), to

produce a third population of TILs, wherein the second expansion is performed for about 7-11 days to obtain the third population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, and wherein the transition from step (b) to step (c) occurs without opening the system;

- (d) harvesting the third population of TILs obtained from step (c), wherein the transition from step (c) to step (d) occurs without opening the system;
- (e) transferring the harvested third TIL population from step (d) to an infusion bag, wherein the transfer from step (d) to (e) occurs without opening the system;
- (f) cryopreserving the infusion bag comprising the harvested TIL population from step (e) using a cryopreservation process; and
- (g) administering a therapeutically effective dosage of the third population of TILs from the infusion bag in step (f) to the subject;

wherein the APCs are selected from the group consisting of Raji, Ramos, Daudi, U937, or Thp1 cells, or a derivative, variant, modification, or progeny thereof.

[0073] In some examples, the cancer is selected from the group consisting of melanoma (including metastatic melanoma and uveal melanoma), ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), small cell lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell carcinoma (HNSCC)), esophageal cancer, esophagogastric junction cancer, gastric cancer, gastrointestinal cancer, renal cancer, and renal cell carcinoma.

[0074] In some examples, the first expansion in step (a) and the second expansion in step (b) are each individually performed within a period of 11 days.

[0075] In some examples, steps (a) through (d) are performed in about 10 days to about 22 days.

[0076] In some examples, the potency of the TILs has been determined using the method as described herein.

[0077] In some examples, the potency of the TILs has been determined using the method as described herein.

WHAT IS CLAIMED IS:

1. A method of determining the potency of a TIL, the method comprising the steps of:
 - a. performing a co-culture of a target cell with a TIL cell for a first period;
 - b. obtaining a harvest or extracting a supernatant from the co-culture; and
 - c. assessing (1) the harvest for expression of one or more markers on the TIL cell or (2) the supernatant for one or more analytes secreted from the TIL cell to obtain one or more observed values to determine the potency for the TIL.
2. The method of Claim 1, the method comprising the additional steps of:
 - d. performing a second co-culture of a negative control comprising: (i) a negative control cell with the target cell, or (ii) a human leukocyte antigen (HLA) blocking antibody with the TIL cell and the target cell, for a second period, such second period optionally occurring simultaneously with the first period;
 - e. obtaining a second harvest or extracting a second supernatant from the second co-culture;
 - f. assessing (1) the second harvest for the expression of the one or more markers on the TIL cell or (2) the second supernatant for the one or more analytes secreted from the TIL cell to obtain one or more control values; and
 - g. comparing the one or more observed values from step c with the one or more control values from step f, where each observed value or set of observed values is compared to a corresponding control value or set of control values, to determine the potency of the TIL.
3. The method of any one of Claims 1 to 2, wherein the TIL is selected from the group consisting of a tumor-infiltrating lymphocyte (TIL) product, a marrow-infiltrating lymphocyte (MIL) product, or a peripheral blood lymphocyte (PBL) product, and wherein the method optionally further comprises the step of thawing a cryopreserved TIL product, MIL product, or PBL product.
4. The method of any one of Claims 1 to 3, wherein the TIL is a TIL product from a human, and wherein the TIL product is obtained by resection of a tumor or fragmentation or digestion of a tumor and manufactured by a TIL expansion process comprising a rapid expansion protocol step.

5. The method of any one of Claims 1 to 4, further comprising the step of releasing the TIL.
6. The method of any one of Claims 1 to 5, wherein the target cell is selected from the group consisting of a monocyte cell, a B-lymphoblastoid cell, a Burkitt's lymphoma cell, a Raji cell, a Thp1 cell, a Ramos cell, a U937 cell, a Daudi cell, and derivatives, variants, modifications, or progeny thereof, and combinations thereof, wherein such target cell or combinations thereof are optionally irradiated, and wherein the target cell or combinations thereof are optionally alloreactive to the TIL, MIL, or PBL product.
7. The method of any one of Claims 2 to 6, wherein the negative control cell lacks MHC or HLA Class I and MHC or HLA Class II expression.
8. The method of Claim 7, wherein the negative control cell is a K562 cell or a derivative, variant, modification, or progeny thereof, wherein such negative control cell is optionally irradiated.
9. The method of any one of Claims 2 to 8, wherein the ratio between the number of TIL product cells to the number of target cells is between 5:1 and 1:5 and wherein the ratio between the number of TIL product cells to the number of negative control cells is between 5:1 and 1:5.
10. The method of any one of Claims 2 to 8, wherein the ratio between the number of TIL product cells to the number of negative control cells is between 1:2 and 1:4 and wherein the ratio between the number of TIL product cells to the number of negative control cells is between 1:2 and 1:4.
11. The method of any one of Claims 1 to 10, wherein the first period is from about 6 hours to about 48 hours.
12. The method of any one of Claims 1 to 11, wherein the second period is from about 6 hours to about 48 hours.
13. The method of any one of Claims 1 to 12, wherein the first period is selected from the group consisting of about 12 hours, about 18 hours, and about 24 hours.
14. The method of any one of Claims 1 to 13, wherein the second period is selected from the group consisting of about 12 hours, about 18 hours, and about 24 hours.
15. The method of any one of Claims 1 to 14, wherein the one or more markers on the TIL are selected from the group consisting of CD25, CD69, CD134, CD137, CD150,

KLRG1, or combinations thereof.

16. The method of any one of Claims 1 to 15, wherein the one or more analytes secreted from the TIL is selected from the group consisting of IFN- α , IFN- β , IFN- γ , granzyme B, perforin, TNF- α , IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-14, IL-16, IL-17, IL-18, IL-22, IL-25, IL-26, MIP-1 β , and combinations thereof.
17. The method of any one of Claims 4 to 15, wherein the one or more analytes secreted from the TIL product is selected from the group consisting of IFN- α , IFN- β , IFN- γ , granzyme B, perforin, TNF- α , IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-14, IL-16, IL-17, IL-18, IL-22, IL-25, IL-26, MIP-1 β , and combinations thereof, wherein the quantity of the observed value is normalized to the quantity of the control value for each of the one or more analytes, and wherein the increase in observed value over the control value for each of the one or more analytes is selected from the group consisting of at least 1-fold, at least 1.5-fold, at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold, at least 4-fold, at least 4.5-fold, and at least 5-fold.
18. The method of any one of Claims 4 to 17, wherein the TIL product is manufactured from a tumor obtained by surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining a sample that contains a mixture of tumor and TIL cells from a human patient.
19. The method of Claim 1, wherein the method is a component of a potency assay matrix.
20. The method of Claim 19, wherein the potency assay matrix comprises one or more assays selected from the group consisting of a bead- or plate-based assay using CD3, CD28, and/or CD137 stimulation and reporting interferon- γ , granzyme B, or tumor necrosis factor- α , an assay for total viable cells, an assay for percentage viable cells, an assay for CD4⁺ cell content, an assay for CD8⁺ cell content, an assay for T_{EM} cell content, an assay for T_{CM} cell content, an assay for LAG3⁺ cell content, and an assay for KLRG1⁺ cell content, an assay for CD101⁺ cell content, an assay for CD69⁺ cell content, an assay for T_{SCM} cell content, an assay for T_{EMRA} cell content, an assay for T_{reg} cell content, an assay for PD-1⁺ cell content, an assay for TIM3⁺ cell content, an assay for CD25⁺ cell content, an assay for CD27⁺ cell content, an assay for CD28⁺ cell content, an assay for CD56⁺ cell content, an assay for CTLA-4⁺ cell content, an assay for TIGIT⁺ cell content, and an assay for CD57⁺ cell content.