effectiveness and scalability in manufacturing and more potent anti-cancer phenotypes of TIL preparations produced for treatment of human patients at multiple clinical centers. The present invention meets this need by providing a novel TIL expansion process which includes antigen-presenting feeder cells from the initiation of expansion, in order to prime the TILs for expansion, rather than a tradition pre-REP expansion step, thus allowing for a substantial reduction in overall time for the expansion process.

SUMMARY

[0004] The present disclosure provides improved and/or shortened methods for expanding TILs and producing therapeutic populations of TILs.

[0005] In one aspect, the present disclosure refers to a method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising: (a) performing a priming first expansion by culturing a first population of TILs, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a subject into multiple tumor fragments, in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of 1 to 7 or 8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs; (b) performing a rapid second expansion by contacting the second population of TILs to a cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; and (c) harvesting the therapeutic population of TILs obtained from step (b).

permeable surface area, wherein the priming first expansion is performed for first period of about 1 to 7/8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;

(c) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the number of APCs added in the rapid second expansion is at least twice the number of APCs added in step (b), wherein the rapid second expansion is performed for a second period of about 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;

(d) harvesting the therapeutic population of TILs obtained from step (c); and

(e) transferring the harvested TIL population from step (d) to an infusion bag.

[0006] In another aspect, the present disclosure refers to expanded tumor infiltrating lymphocytes (TILs) for use in a method of treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising: (a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments; (b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for 1 to 7 or 8 days to obtain the second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs; (c) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; (d) harvesting the therapeutic population of TILs obtained from step (c); (e) transferring the harvested TIL population from step (d) to an infusion bag; and (f) administering a therapeutically effective dosage of the TILs from step (e) to the subject.

WHAT IS CLAIMED IS:

1. A method for expanding tumor infiltrating lymphocytes (TILs) into a therapeutic population of TILs comprising:
    (a) performing a priming first expansion by culturing a first population of TILs, said first population of TILs obtainable by processing a tumor sample from a tumor resected from a subject into multiple tumor fragments, in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for first period of 1 to 7 or 8 days to obtain the second population of TILs, wherein the second population of TILs is greater in number than the first population of TILs;
    (b) performing a rapid second expansion by contacting the second population of TILs to a cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for a second period of 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area; and
    (c) harvesting the therapeutic population of TILs obtained from step (b).

2. The method of claim 1, wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is 1.5:1 to 100:1, optionally wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is 50:1, further optionally wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is 25:1, further optionally wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is 20:1 and further optionally wherein the ratio of the number of TILs in the second population of TILs to the number of TILs in the first population of TILs is 10:1, wherein optionally the second population of TILs is at least 50-fold greater in number than the

first population of TILs, wherein further optionally the method comprises performing, after the step of harvesting the therapeutic population of TILs, the additional step of:

transferring the harvested therapeutic population of TILs to an infusion bag.

3. The method of any of claims 1 to 2, wherein the multiple tumor fragments are distributed into a plurality of separate containers, in each of which separate containers the second population of TILs is obtained from the first population of TILs in the step of the priming first expansion, and the third population of TILs is obtained from the second population of TILs in the step of the rapid second expansion, and wherein the therapeutic population of TILs obtained from the third population of TILs is collected from each of the plurality of containers and combined to yield the harvested TIL population, wherein optionally the plurality of separate containers comprises at least two separate containers, e.g. from two to twenty separate containers e.g. from two to ten separate containers e.g. two to five separate containers, wherein further optionally each of the separate containers comprises a first gas-permeable surface area.

4. The method of any of claims 1 to 3, wherein the multiple tumor fragments are distributed in a single container, wherein optionally the single container comprises a first gas-permeable surface area.

5. The method of claim 3 or claim 4, wherein in the step of the priming first expansion the cell culture medium comprises antigen-presenting cells (APCs) and the APCs are layered onto the first gas-permeable surface area at an average thickness of one cell layer to three cell layers, e.g. 1.5 cell layers to 2.5 cell layers, e.g. 2 cell layers.

6. The method of any of claims 1-3, wherein in the step of the priming first expansion the priming first expansion is performed in a first container comprising a first gas-permeable surface area and in the step of the rapid second expansion the rapid second expansion is performed in a second container comprising a second gas-permeable surface area, wherein optionally the second container is larger than the first container.

7. The method of claim 6, wherein in the step of the rapid second expansion the APCs are layered onto the second gas-permeable surface area at an average thickness of 3 cell layers to 5 cell layers.

8. The method of any of claims 1 to 5, wherein for each container in which the priming first expansion is performed on a first population of TILs the rapid second expansion is performed in the same container on the second population of TILs produced from such first population of TILs, wherein optionally each container comprises a first gas-permeable surface area.

9. The method of any of claims 1-5, 6 and 8, wherein for each container in which the priming first expansion is performed on a first population of TILs in the step of the priming first expansion the first container comprises a first surface area, the cell culture medium comprises antigen-presenting cells (APCs), and the APCs are layered onto the first gas-permeable surface area, and wherein the number of APCs in the rapid expansion step is at least twice the number of APCs in the priming first expansion step.

10. The method of any of the preceding claims, wherein after 2 to 3 days in the step of the rapid second expansion, the cell culture medium is supplemented with additional IL-2,

    wherein optionally further comprising cryopreserving the harvested TIL population in the step of harvesting the therapeutic population of TILs using a cryopreservation process.

11. The method according to claim 3, further comprising the step of cryopreserving the infusion bag optionally wherein the cryopreservation process is performed using a 1:1 ratio of harvested TIL population to cryopreservation media.

12. The method according to any of the preceding claims, wherein the antigen-presenting cells are peripheral blood mononuclear cells (PBMCs), wherein optionally the PBMCs are irradiated and allogeneic, wherein further optionally in the step of the priming first expansion the cell culture medium comprises peripheral blood mononuclear cells

(PBMCs), and wherein the total number of PBMCs added to the cell culture medium in the step of the priming first expansion is 2.5×10^8 and/or wherein in the step of the rapid second expansion the antigen-presenting cells (APCs) in the cell culture medium are peripheral blood mononuclear cells (PBMCs), and wherein the total number of PBMCs added to the cell culture medium in the step of the rapid second expansion is 5×10^8.

13. The method according to any of claims 1-12, wherein the antigen-presenting cells are artificial antigen-presenting cells.

14. The method according to any of the preceding claims, wherein the harvesting in the step of harvesting the therapeutic population of TILs is performed using a membrane-based cell processing system and optionally a LOVO cell processing system.

15. The method according to any of the preceding claims, wherein the multiple fragments comprise 30 to 60 fragments with a total volume of 1300 mm^3 to 1500 mm^3, wherein optionally the multiple fragments comprise 60 fragments per container in the step of the priming first expansion, wherein each fragment has a volume of 27 mm^3, wherein further optionally the multiple fragments have a total mass of 1 gram to 1.5 grams.

16. The method according to any of the preceding claims, wherein the cell culture medium is provided in a container selected from the group consisting of a G-container and a Xuri cellbag.

17. The method according to any of the preceding claims, wherein the IL-2 concentration is 10,000 IU/mL to 5,000 IU/mL.

18. The method according to any of claims 11-13, wherein the cryopreservation media comprises dimethlysulfoxide (DMSO), wherein optionally the cryopreservation media comprises 7% to 10% DMSO.

19. The method according to any of the preceding claims, wherein the first period in the step of the priming first expansion is performed within a period of 5 days, 6 days, or 7 days, and wherein the second period in the step of the rapid second expansion is performed within a period of 5 days, 6 days, 7 days, 8 days, or 9 days.

20. The method according to any of claims 1-18, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs are performed within a period of 14 days to 16 days, e.g. within a period of 16 days, e.g. within a period of 15 days, e.g. within a period of 14 days.

21. The method according to any of claims 1-18, further comprising the step of cryopreserving the harvested therapeutic population of TILs using a cryopreservation process, wherein steps of the priming first expansion through the harvesting of the therapeutic population of TILs and cryopreservation are performed in 16 days or less.

22. The method according to any one of claims 1 to 20, wherein the therapeutic population of TILs harvested in the step of harvesting of the therapeutic population of TILs comprises sufficient TILs for a therapeutically effective dosage of the TILs,

    wherein optionally the number of TILs sufficient for a therapeutically effective dosage is from 2.3×10^{10} to 13.7×10^{10}.

23. The method according to any one of claims 1 to 22, wherein the third population of TILs in the step of the rapid second expansion provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality,

    wherein optionally the third population of TILs in the step of the rapid second expansion provides for at least a one-fold to five-fold or more interferon-gamma production as compared to TILs prepared by a process longer than 18 days, wherein further optionally the effector T cells and/or central memory T cells obtained from the third population of TILs in the step of the rapid second expansion exhibit increased CD8 and CD28 expression relative to effector T cells and/or central memory T cells obtained from the second population of TILs in the step of the priming first expansion.

24. The method according to any one of claims 1 to 23, wherein the therapeutic population of TILs from the step of the harvesting of the therapeutic population of TILs are to be infused into a patient.

25. Expanded tumor infiltrating lymphocytes (TILs) for use in a method of treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:
    (a) obtaining and/or receiving a first population of TILs from a tumor resected from a subject by processing a tumor sample obtained from the subject into multiple tumor fragments;
    (b) performing a priming first expansion by culturing the first population of TILs in a cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs) to produce a second population of TILs, wherein the priming first expansion is performed in a container comprising a first gas-permeable surface area, wherein the priming first expansion is performed for 1 to 7 or 8 days to obtain the second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs;
    (c) performing a rapid second expansion by supplementing the cell culture medium of the second population of TILs with additional IL-2, OKT-3, and APCs, to produce a third population of TILs, wherein the rapid second expansion is performed for 1 to 11 days to obtain the third population of TILs, wherein the third population of TILs is a therapeutic population of TILs, wherein the rapid second expansion is performed in a container comprising a second gas-permeable surface area;
    (d) harvesting the therapeutic population of TILs obtained from step (c);
    (e) transferring the harvested TIL population from step (d) to an infusion bag; and
    (f) administering a therapeutically effective dosage of the TILs from step (e) to the subject.

26. Expanded tumor infiltrating lymphocytes (TILs) for use according to claim 25, wherein the number of TILs sufficient for administering a therapeutically effective dosage in step (f) is from 2.3×10^{10} to 13.7×10^{10}.

27. Expanded tumor infiltrating lymphocytes (TILs) for use according to claim 25, wherein the antigen presenting cells (APCs) are PBMCs, wherein optionally prior to administering a therapeutically effective dosage of TIL cells in step (f), a non-myeloablative lymphodepletion regimen has been administered to the patient, wherein further optionally the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days.

28. Expanded tumor infiltrating lymphocytes (TILs) for use according to any of claims 25 to 27, further comprising the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the TIL cells to the patient in step (f), wherein optionally the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

29. Expanded tumor infiltrating lymphocytes (TILs) for use according to any one of claims 25 to 27, wherein the third population of TILs in step (c) provides for increased efficacy, increased interferon-gamma production, and/or increased polyclonality, wherein optionally the third population of TILs in step (c) provides for at least a one-fold to five-fold or more interferon-gamma production as compared to TILs prepared by a process longer than 16 days, wherein further optionally the effector T cells and/or central memory T cells obtained from the third population of TILs in step (c) exhibit increased CD8 and CD28 expression relative to effector T cells and/or central memory T cells obtained from the second population of cells in step (b).

30. Expanded tumor infiltrating lymphocytes (TILs) for use according to any one of claims 25 to 28, wherein the cancer is a solid tumor, optionally wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer (including head and neck squamous cell

carcinoma (HNSCC)), glioblastoma (including GBM), gastrointestinal cancer, renal cancer, and renal cell carcinoma.

31. Expanded tumor infiltrating lymphocytes (TILs) for use according to any one of claims 25 to 30, wherein the container is a closed container, wherein optionally the closed container comprises a GREX-100 or a GREX-500; a G-container or a GREX-10.

32. The method of any of claims 1 to 24 wherein the tumor sample is one or more small biopsies, core biopsies, or needle biopsies of the tumor in the subject.