[004] Direct enzymatic digestion of resected tumors has been previously explored as an alternative to pre-REP, but has been reported to yield less TIL cultures, resulting in a decreased ability to obtain TILs than from pre-REP initiation processes with IL-2. Dudley, *et al.*, *J. Immunother.* **2003,** *26,* 332-42. For this reason, digestion has not been further explored in the development of TILs as a therapy for cancer.

[005] TILs obtained from the pre-REP and REP processes have dominated the clinical studies of TILs to date, which have offered modest clinical responses, and the field remains challenging, particularly in the extension of TIL therapy from melanoma to other tumor types. Goff, *et al., J. Clin. Oncol.* **2016,** *34,* 2389-97; Dudley, *et al.*, *J. Clin. Oncol.* **2008**, *26,* 5233-39; Rosenberg, *et al.*, *Clin. Cancer Res.* **2011,** *17,* 4550-57. Much focus has been placed on selection of TILs during expansion to either select particular subsets (such as $CD8^+$ T cells) or to target driver mutations such as a mutated ERBB2IP epitope or driver mutations in the KRAS oncogene. Tran, *et al.*, *N. Engl. J. Med.* **2016,** *375,* 2255-62; Tran, *et al., Science* **2014,** *344,* 641-45. However, such selection approaches, even if they can be developed to show efficacy in larger clinical trials, add significantly to the duration, complexity, and cost of performing TIL therapy and limit the potential for widespread use of TIL therapy in different types of cancers. Thus, there is an urgent need to develop processes capable of providing TILs with improved properties for use in cancer therapies.

[006] The invention provides the unexpected finding that TILs with improved properties may be obtained from processes based on tumor remnant cells, and that such remnant TILs (rTILs) are phenotypically and functionally distinct from normal emigrant TILs (eTILs). The use of rTILs and combinations of rTILs and eTILs in cancer immunotherapy provides significant advantages over prior eTIL-based therapies.

SUMMARY OF THE INVENTION

[006a] In one aspect, the present invention refers to method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising: (a) fragmenting tumor tissue obtained from a patient, wherein wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs); (b) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs); (c) removing at least a plurality of the eTILs; (d) enzymatically digesting the

tumor remnants into tumor remnant cells using a digest mixture; and (e) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs); wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs, and wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof. In another aspect, the present invention refers to the use of a therapeutically effective amount of rTILs in the manufacture of a medicament for treating cancer, wherein the rTILs are prepared according to the method of the present invention. In another aspect, the present invention refers to the use of a therapeutically effective amount of rTILs in the manufacture of a medicament for treating cancer in a patient, wherein the use comprises obtaining rTILs from a tumor resected from a patient according to the method of the present invention; wherein the patient is to be treated with a non-myeloablative lymphodepletion regimen prior to administration of the rTILs; wherein the therapeutically effective amount of rTILs may be optionally cryopreserved and thawed prior to the administration to the patient; and wherein the patient is to be treated with a high-dose IL-2 regimen starting on the day after administration of the rTILs.

[007] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable

container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs, and wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof.

[008] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof, and

wherein the tumor tissue is selected from the group consisting of melanoma tumor tissue, head and neck tumor tissue, breast tumor tissue, renal tumor tissue, pancreatic tumor tissue, glioblastoma tumor tissue, lung tumor tissue, colorectal tumor tissue, sarcoma tumor tissue, triple negative breast tumor tissue, cervical tumor tissue, ovarian tumor tissue, and acute myeloid leukemia bone marrow or tumor tissue.

[009] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof, and

wherein the irradiated feeder cells comprise irradiated allogeneic peripheral blood mononuclear cells.

[0010] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein IL-2 is present in the second cell culture medium at an initial concentration of about 3000 IU/mL and OKT-3 antibody is present in the second cell culture medium at an initial concentration of about 30 ng/mL.

[0011] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein at least one T cell exhaustion marker in $CD8^+$ and $CD4^+$ T cells in the rTILs is reduced by at least 10% relative to the eTILs.

[0012] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the T cell exhaustion marker is a LAG3 marker in $CD8^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 2-fold relative to the eTILs.

[0013] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the T cell exhaustion marker is a TIM3 marker in $CD8^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 3-fold relative to the eTILs.

[0014] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the T cell exhaustion marker is a TIM3 marker in $CD4^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 2-fold relative to the eTILs.

[0015] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the TIM3 marker and the LAG3 marker in the rTILs are undetectable by flow cytometry.

[0016] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein $CD56^+$ expression in the rTILs is reduced by at least 3-fold relative to $CD56^+$ expression in the eTILs.

[0017] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein $CD69^+$ expression in the rTILs is increased by at least 2-fold relative to $CD69^+$ expression in the eTILs.

[0018] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the digest mixture comprises deoxyribonuclease, collagenase, and hyaluronidase.

[0019] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0020] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0021] In an example, also described herein is a method of treating a cancer in a patient in need of such treatment, wherein the treatment comprises delivering a therapeutically effective amount of rTILs to a patient, wherein the rTILs are prepared according a method comprising the steps of:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0022] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient.

[0023] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient.

[0024] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient,

wherein the cancer is selected from the group consisting of melanoma, double-refractory melanoma, ovarian cancer, cervical cancer, lung cancer, bladder cancer, breast cancer, head and neck cancer, renal cell carcinoma, acute myeloid leukemia, colorectal cancer, sarcoma, non-small cell lung cancer (NSCLC), and triple negative breast cancer.

[0025] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient, wherein 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;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient.

[0026] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing at least a plurality of the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient,

wherein the high-dose IL-2 regimen comprises 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.

[0027] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs, and wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof.

[0028] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof, and

wherein the tumor tissue is selected from the group consisting of melanoma tumor tissue, head and neck tumor tissue, breast tumor tissue, renal tumor tissue, pancreatic tumor tissue, glioblastoma tumor tissue, lung tumor tissue, colorectal tumor tissue, sarcoma tumor tissue, triple negative breast tumor tissue, cervical tumor tissue, ovarian tumor tissue, and acute myeloid leukemia bone marrow or tumor tissue.

[0029] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof, and

wherein the irradiated feeder cells comprise irradiated allogeneic peripheral blood mononuclear cells.

[0030] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein IL-2 is present in the second cell culture medium at an initial concentration of about 3000 IU/mL and OKT-3 antibody is present in the second cell culture medium at an initial concentration of about 30 ng/mL.

[0031] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein at least one T cell exhaustion marker in $CD8^+$ and $CD4^+$ T cells in the rTILs is reduced by at least 10% relative to the eTILs.

[0032] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the T cell exhaustion marker is a LAG3 marker in $CD8^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 2-fold relative to the eTILs.

[0033] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the T cell exhaustion marker is a TIM3 marker in $CD8^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 3-fold relative to the eTILs.

[0034] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the T cell exhaustion marker is a TIM3 marker in $CD4^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 2-fold relative to the eTILs.

[0035] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the TIM3 marker and the LAG3 marker in the rTILs are undetectable by flow cytometry.

[0036] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein $CD56^+$ expression in the rTILs is reduced by at least 3-fold relative to $CD56^+$ expression in the eTILs.

[0037] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein $CD69^+$ expression in the rTILs is increased by at least 2-fold relative to $CD69^+$ expression in the eTILs.

[0038] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the digest mixture comprises deoxyribonuclease, collagenase, and hyaluronidase.

[0039] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0040] In an example, also described herein is a method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0041] In an example, also described herein is a method of treating a cancer in a patient in need of such treatment, wherein the treatment comprises delivering a therapeutically effective amount of rTILs to a patient, wherein the rTILs are prepared according a method comprising the steps of:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable

container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

[0042] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient.

[0043] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient.

[0044] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient,

wherein the cancer is selected from the group consisting of melanoma, double-refractory melanoma, ovarian cancer, cervical cancer, lung cancer, bladder cancer, breast cancer, head and neck cancer, renal cell carcinoma, acute myeloid leukemia, colorectal cancer, sarcoma, non-small cell lung cancer (NSCLC), and triple negative breast cancer.

[0045] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient, wherein 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;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient.

[0046] In an example, also described herein is a method treating a cancer in a patient in need of such treatment, the method comprising:

(a) obtaining tumor tissue from the patient, wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

(b) fragmenting the tumor tissue;

(c) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

(d) removing the eTILs;

(e) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture;

(f) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs,

wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, and combinations thereof, and

wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof,

(g) treating the patient with a non-myeloablative lymphodepletion regimen prior to administering the rTILs to the patient;

(h) administering a therapeutically effective amount of rTILs to the patient, wherein a therapeutically effective amount of eTILs are simultaneously administered to the patient in a mixture with the rTILs; and

(i) treating the patient with a high-dose IL-2 regimen starting on the day after administration of the rTILs to the patient,

wherein the high-dose IL-2 regimen comprises 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.

[0047] In an example, also described herein is a process for generating an expanded number of tumor remnant cells that include tumor infiltrating lymphocytes (TILs) from a patient for adoptive T cell therapy. In some examples, the process of the disclosure may include the step of obtaining tumor tissue from the patient, wherein the tumor tissue comprises TILs. In some examples, the process of the disclosure may include the step of fragmenting the tumor tissue. In some examples, the process of the disclosure may include the step of treating the tumor tissue in a gas permeable container with cell culture media and interleukin 2 (IL-2) and other T cell growth factors or agonistic antibodies to provide tumor remnants and an expanded number of TILs. In some examples, the process of the disclosure may include the step of removing the expanded number of TILs. In some examples, the process of the disclosure may include the step of enzymatically digesting the tumor remnants into tumor remnant cells. In some examples, the process of the disclosure may include the step of treating the tumor remnant cells with cell culture media, irradiated feeder cells, anti-CD3 monoclonal antibody (muromonab or OKT-3),

and IL-2 to provide the expanded number of tumor remnant cells. In some examples, the tumor remnant cells prepared according to the processes of the disclosure may include TILs that express reduced levels of at least one marker selected from the group consisting of TIM3, LAG3, PD-1, and combinations thereof. In some examples, the tumor tissue may be selected from the group consisting of melanoma tumor tissue, head and neck tumor tissue, breast tumor tissue, renal tumor tissue, pancreatic tumor tissue, lung tumor tissue, and colorectal tumor tissue.

[0048] In an example, the disclosure may include method of treating a tumor in a patient in need of such treatment. In some examples, the treatment may include delivering a therapeutically effective amount of an expanded number of tumor remnant cells to the patient, wherein the expanded number of tumor remnant cells may be prepared according to any process described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

[0050] FIG. 1 illustrates an exemplary diagram of the tumor digestion solution preparation.

[0051] FIG. 2 illustrates an exemplary flow-through diagram of the tumor digestion procedure. In this example, two digestion methods are performed simultaneously and are seeded separately as a part of two distinct pre-REPs designed to compare the efficacy of each digestion method.

[0052] FIG. 3 illustrates differential phenotypic expression of key markers in eTILs and rTILs.

CLAIMS

We claim:

1. A method for preparing remnant tumor infiltrating lymphocytes (rTILs) for adoptive T cell therapy, the method comprising:

   (a) fragmenting tumor tissue obtained from a patient, wherein wherein the tumor tissue comprises tumor infiltrating lymphocytes (TILs);

   (b) treating the tumor tissue in a gas permeable container with a first cell culture medium and interleukin 2 (IL-2) to provide tumor remnants and emergent TILs (eTILs);

   (c) removing at least a plurality of the eTILs;

   (d) enzymatically digesting the tumor remnants into tumor remnant cells using a digest mixture; and

   (e) expanding the tumor remnant cells with a second cell culture medium comprising cell culture media, irradiated feeder cells, OKT-3 antibody, and IL-2 in a gas permeable container to provide an expanded number of remnant tumor infiltrating lymphocytes (rTILs);

   wherein the rTILs express reduced levels of a T cell exhaustion marker relative to the eTILs, and wherein the T cell exhaustion marker is selected from the group consisting of TIM3, LAG3, TIGIT, PD-1, CTLA-4, and combinations thereof.

2. The method of Claim 1, wherein the tumor tissue is selected from the group consisting of melanoma tumor tissue, head and neck tumor tissue, breast tumor tissue, renal tumor tissue, pancreatic tumor tissue, glioblastoma tumor tissue, lung tumor tissue, colorectal tumor tissue, sarcoma tumor tissue, triple negative breast tumor tissue, cervical tumor tissue, ovarian tumor tissue, and acute myeloid leukemia bone marrow or tumor tissue.

3. The method of Claim 1 or 2, wherein the irradiated feeder cells comprise irradiated allogeneic peripheral blood mononuclear cells.

4. The method of any one of Claims 1 to 3, wherein IL-2 is present in the second cell culture medium at an initial concentration of about 3000 IU/mL and OKT-3 antibody is present in the second cell culture medium at an initial concentration of about 30 ng/mL.

5. The method of any one of Claims 1 to 4, wherein at least one T cell exhaustion marker in $CD8^+$ and $CD4^+$ T cells in the rTILs is reduced by at least 10% relative to the eTILs.

6. The method of any one of Claims 1 to 5, wherein the T cell exhaustion marker is a LAG3 marker in $CD8^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 2-fold relative to the eTILs.

7. The method of any one of Claims 1 to 6, wherein the T cell exhaustion marker is a TIM3 marker in $CD8^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 3-fold relative to the eTILs.

8. The method of any one of Claims 1 to 7, wherein the T cell exhaustion marker is a TIM3 marker in $CD4^+$ T cells, and wherein the LAG3 marker in the rTILs is reduced by at least 2-fold relative to the eTILs.

9. The method of any one of Claims 1 to 8, wherein the TIM3 marker and the LAG3 marker in the rTILs are undetectable by flow cytometry.

10. The method of any one of Claims 1 to 9, wherein $CD56^+$ expression in the rTILs is reduced by at least 3-fold relative to $CD56^+$ expression in the eTILs.

11. The method of any one of Claims 1 to 10, wherein $CD69^+$ expression in the rTILs is increased by at least 2-fold relative to $CD69^+$ expression in the eTILs.

12. The method of any one of Claims 1 to 11, wherein the digest mixture comprises deoxyribonuclease, collagenase, and hyaluronidase.

13. The method of any one of Claims 1 to 12, wherein the first cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

14. The method of any one of Claims 1 to 13, wherein the second cell culture medium further comprises a cytokine selected from the group consisting of IL-4, IL-7, IL-15, IL-21, and combinations thereof.

15. The method of any one of Claims 1 to 14, further comprising the steps of cryopreserving the expanded number of rTILs to form a cryopreserved rTIL population.

16. The method of Claim 15, further comprising the step of thawing the cryopreserved rTIL population.

17. The method of any one of Claims 1 to 16, wherein the rTILs are substantially free of eTILs.

18. The method of any one of Claims 1 to 17, wherein the step of expanding the tumor remnant cells with the second cell culture medium comprises adding eTILs to the second cell culture medium to provide a selected rTIL to eTIL ratio.

19. The method of any one of Claims 1 to 17, further comprising the step of adding eTIL to the rTIL to provide a selected rTIL to eTIL ratio.

20. The method of Claim 18 or 19, wherein the selected rTIL to eTIL ratio is at least 1% rTIL to eTIL.

21. The method of Claim 20, wherein the selected rTIL to eTIL ratio is at most 99.9% rTIL to eTIL.

22. The method of Claim 18 or 19, wherein the selected rTIL to eTIL ratio is selected from the group consisting of about 1:99, about 5:95, about 10:90, about 15:85, about 20:80, about 25:75, about 30:70, about 35:65, about 40:60, about 45:55, about 50:50, about 55:45, about 60:40, about 65:35, about 70:30, about 75:25, about 80:20, about 85:15, about 90:10, about 95:5, and about 99:1 rTIL to eTIL.

23. Use of a therapeutically effective amount of rTILs in the manufacture of a medicament for treating cancer, wherein the rTILs are prepared according to the method of any one of Claims 1 to 22.

24. Use of a therapeutically effective amount of rTILs in the manufacture of a medicament for treating cancer in a patient, wherein the use comprises obtaining rTILs from a tumor resected from a patient according to the method of any one of Claims 1 to 22; wherein the patient is to be treated with a non-myeloablative lymphodepletion regimen prior to administration of the rTILs; wherein the therapeutically effective amount of rTILs may be optionally cryopreserved and thawed prior to the administration to the patient; and wherein the patient is to be treated with a high-dose IL-2 regimen starting on the day after administration of the rTILs.

25. The use of any one of Claims 23 to 24, wherein a therapeutically effective amount of eTILs are to be simultaneously administered to the patient in a mixture with the rTILs.

26. The use of any one of Claims 23 to 25, wherein the non-myeloablative lymphodepletion regimen comprises that cyclophosphamide is to be administered 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.

27. The use of any one of Claims 23 to 26, wherein the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg of aldesleukin, or a biosimilar or variant thereof, to be administered as a 15-minute bolus intravenous infusion every eight hours until tolerance.

28. The use of any one of Claims 23 to 27, wherein the cancer is selected from the group consisting of melanoma, double-refractory melanoma, uveal melanoma, ovarian cancer, cervical cancer, lung cancer, bladder cancer, breast cancer, head and neck cancer, renal cell carcinoma, acute myeloid leukemia, colorectal cancer, and sarcoma.

29. The use of Claim 28, wherein the cancer is breast cancer, and wherein the breast cancer is selected from the group consisting of triple negative breast cancer, estrogen receptor-positive breast cancer, progesterone receptor-positive breast cancer, and estrogen receptor-positive/progesterone receptor-positive breast cancer.

30. The use of Claim 28, wherein the cancer is lung cancer, and the lung cancer is selected from the group consisting of non-small cell lung cancer and small cell lung cancer.