

SUMMARY

[0004a] In one aspect, the present disclosure relates to a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising: (a) culturing a first population of TILs obtained and/or received from a tumor tissue resected from a subject or patient in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs; (b) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 agonist and anti-CD28 agonist beads or antibodies, for 1-7 days, to produce a third population of TILs; (c) gene-editing at least a portion of the third population of TILs, to produce a fourth population of TILs; and (d) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

[0004b] In another aspect, the present disclosure relates to a gene-edited population of tumor infiltrating lymphocytes (TILs) comprising an expanded population of TILs wherein the expression of at least one protein is modulated by a gene editor transferred into at least a portion of the expanded population of TILs.

[0004c] In another aspect, the present disclosure relates to a therapeutically effective dose of the gene edited population of TILs as disclosed herein for use in therapy.

[0004d] In another aspect, the present disclosure relates to use of a therapeutically effective dose of the gene edited population of tumor infiltrating lymphocytes (TILs) of any one of claims 13-15 in the manufacture of a medicament for treating cancer, wherein the cancer is selected from the group consisting of melanoma, metastatic melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), metastatic 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)), renal cancer, and renal cell carcinoma. **[0004e]** In another aspect, the present disclosure relates to use of an expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating cancer, the use comprises: (a) obtaining a first population of TILs from a patient by processing a tumor sample obtained from the patient into multiple tumor fragments; (b) performing a first expansion by culturing the first population of TILs in a first 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 3-8 days to obtain the second population of TILs; (c) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 and anti-CD28 agonist beads or antibodies, for 1-6 days, to produce a third population of TILs; (e) performing a sterile electroporation step on the third population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor; (f) resting the third population of TILs for about 1 day; (g) performing a second expansion by culturing the third population of TILs in a second cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs), to produce a fourth population of TILs,

wherein the second expansion is performed for about 5-15 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, wherein the fourth population of TILs is a therapeutic population of TILs; (h) harvesting the therapeutic population of TILs obtained from step (e) to provide a harvested TIL population; (i) transferring the harvested TIL population to an infusion bag; (j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium; and (k) a therapeutically effective dosage of the harvested TIL population from the infusion bag to be administered to the patient; wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector Nuclease (TALEN) system for inhibiting the expression of PD-1, CTLA-4, LAG-3, CISH, TIGIT and/or CBL-B.

[0005] Provided herein are methods for expanding TILs and producing therapeutic populations of TILs, including methods for gene-editing at least a portion of the TILs to enhance their therapeutic efficacy.

[0006] In some embodiments, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;
- (b) culturing the first population of TILs in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;
- (c) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 agonist and anti-CD28 agonist beads or antibodies, for 1-7 days, to produce a third population of TILs;
- (d) gene-editing at least a portion of the third population of TILs, to produce a fourth population of TILs; and
- (e) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

[0007] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) culturing a first population of TILs obtained and/or received from a tumor tissue resected from a subject or patient in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;
- (b) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 agonist and anti-CD28 agonist beads or antibodies, for 1-7 days, to produce a third population of TILs;
- (c) gene-editing at least a portion of the third population of TILs, to produce a fourth population of TILs; and
- (d) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

[0008] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;
- (b) performing an initial expansion (or priming first expansion) of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first cell culture medium comprises IL-2, optionally OKT-3, and optionally antigen presenting cells (APCs), wherein the priming first expansion occurs for a period of about 3 to 8 days;
- (c) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 agonist and anti-CD28 agonist beads or antibodies, for 1-6 days, to produce a third population of TILs;
- (d) gene-editing at least a portion of the third population of TILs, to produce a fourth population of TILs;
- (e) performing a rapid second expansion of the fourth population of TILs in a second cell culture medium to obtain an expanded number of TILs, wherein the second cell culture medium comprises IL-2, OKT-3, and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second expansion can proceed for about 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days after initiation of the rapid second expansion.

[0009] In some examples, provided herein is a method of expanding tumor infiltrating lymphocytes into a therapeutic population of TILs, the method comprising the steps of:

- (a) obtaining and/or receiving a first population of TILs from a sample of tumor tissue produced by surgical resection, needle biopsy, core biopsy, small biopsy, or other means for obtaining tumor tissue from a patient or subject;
- (b) adding the tumor tissue into a closed system and performing a first expansion by culturing the first population of TILs in a first 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 3-9 days to obtain the second population of TILs;
- (c) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or CD3 agonist and CD28 agonist beads or antibodies, for 1-7 days, to produce a third population of TILs;

(d) gene-editing at least a portion of the third population of TILs, to produce a fourth population of TILs;

(e) performing a second expansion by culturing the fourth population of TILs in a second cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs), to produce a fifth population of TILs, wherein the second expansion is performed for about 5-15 days to obtain the fifth population of TILs, wherein the second expansion is performed in a closed container providing a second gas-permeable surface area, wherein the fifth population of TILs is a therapeutic population of TILs; and

(f) harvesting the therapeutic population of TILs obtained from step (e), wherein each of steps (b) to (f) is performed in a closed, sterile system, and wherein the transition from step (b) to step (c), the transition from step (c) to step (d), the transition from step (d) to step (e) and/or the transition from step (e) to step (f) occurs without opening the system.

[0010] In some examples, the method further comprises:

[0011] digesting in an enzyme media the tumor tissue to produce a tumor digest.

[0012] In some examples, the enzymatic media comprises a DNase.

[0013] In some examples, the enzymatic media comprises a collagenase.

[0014] In some examples, the enzymatic media comprises a neutral protease.

[0015] In some examples, the enzymatic media comprises a hyaluronidase.

[0016] In some examples, the step of culturing or rapid second expansion of the fourth population of TILs is performed by culturing the fourth population of TILs in the second cell culture medium for a first period of about 1-7 days, at the end of the first period the fourth population of TILs is split into a plurality of subcultures, each of the subcultures is cultured in a third cell culture medium comprising IL-2 for a second period of about 3-7 days, and at the end of the second period the subcultures are combined to provide the expanded number of TILs or the therapeutic population of TILs.

[0017] In some examples, the first period of culturing is about 5 days.

[0018] In some examples, the second period of culturing is about 4 days.

[0019] In some examples, the second period of culturing is about 5 days.

[0020] In some examples, the step of activating the second population of TILs is performed using anti-CD3 agonist beads or antibodies.

[0021] In some examples, the step of activating the second population of TILs is performed using OKT-3.

[0022] In some examples, the step of activating the second population of TILs is performed using OKT-3 at 300 ng/mL.

[0023] In some examples, the step of activating the second population of TILs is performed using anti-CD3 agonist and anti-CD28 agonist beads or antibodies.

[0024] In some examples, the step of activating the second population of TILs is performed using TransAct.

[0025] In some examples, the step of activating the second population of TILs is performed using TransAct at 1:10, 1:17.5 or 1:100 dilution.

[0026] In some examples, the step of activating the second population of TILs is performed for about 2 days.

[0027] In some examples, the step of activating the second population of TILs is performed for about 3 days.

[0028] In some examples, the step of activating the second population of TILs is performed for about 4 days.

[0029] In some examples, the step of activating the second population of TILs is performed for about 5 days.

[0030] In some examples, the step of culturing the first population of TILs is performed for about 3 days.

[0031] In some examples, the step of culturing the first population of TILs is performed for about 5 days.

[0032] In some examples, the step of culturing the first population of TILs is performed for about 7 days.

[0033] In some examples, the step of culturing the fourth population of TILs is performed for about 8 days.

[0034] In some examples, the step of culturing the fourth population of TILs is performed for about 9 days.

[0035] In some examples, the step of culturing the fourth population of TILs is performed for about 8-9 days.

[0036] In some examples, the step of culturing the fourth population of TILs is performed for about 10 days.

[0037] In some examples, the step of culturing the fourth population of TILs is performed for about 8-10 days.

[0038] In some examples, all steps are completed within a period of about 22 days.

[0039] In some examples, all steps are completed within a period of about 19-22 days.

[0040] In some examples, all steps are completed within a period of about 19-20 days.

[0041] In some examples, all steps are completed within a period of about 20-22 days.

[0042] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;
- (b) culturing the first population of TILs in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;
- (c) gene-editing at least a portion of the second population of TILs, to produce a third population of TILs; and
- (d) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

[0043] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) culturing a first population of TILs obtained and/or received from a tumor tissue resected from a subject or patient in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;
- (b) gene-editing at least a portion of the second population of TILs, to produce a third population of TILs; and
- (c) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

[0044] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) obtaining and/or receiving a first population of TILs from a tumor tissue resected from a subject or patient;

- (b) performing an initial expansion (or priming first expansion) of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first cell culture medium comprises IL-2, optionally OKT-3, and optionally antigen presenting cells (APCs), wherein the priming first expansion occurs for a period of about 3 to 8 days;
- (c) gene-editing at least a portion of the second population of TILs, to produce a third population of TILs; and
- (d) performing a rapid second expansion of the third population of TILs in a second cell culture medium to obtain an expanded number of TILs, wherein the second cell culture medium comprises IL-2, OKT-3, and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second expansion can proceed for about 1 day, 2 days, 3 days, 4, days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days after initiation of the rapid second expansion.

[0045] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) performing an initial expansion (or priming first expansion) of a first population of TILs obtained and/or received from a tumor tissue resected from a subject or patient in a first cell culture medium to obtain a second population of TILs, wherein the first cell culture medium comprises IL-2, optionally OKT-3, and optionally antigen presenting cells (APCs), wherein the priming first expansion occurs for a period of about 3 to 8 days;
- (b) gene-editing at least a portion of the second population of TILs, to produce a third population of TILs; and
- (c) performing a rapid second expansion of the third population of TILs in a second cell culture medium to obtain an expanded number of TILs, wherein the second cell culture medium comprises IL-2, OKT-3, and APCs; and wherein the rapid expansion is performed over a period of 14 days or less, optionally the rapid second expansion can proceed for about 1 day, 2 days, 3 days, 4, days, 5 days, 6 days, 7 days, 8 days, 9 days or 10 days after initiation of the rapid second expansion.

[0046] In some examples, the method further comprises:

[0047] digesting in an enzyme media the tumor tissue to produce a tumor digest.

[0048] In some examples, the enzymatic media comprises a DNase.

[0049] In some examples, the enzymatic media comprises a collagenase.

[0050] In some examples, the enzymatic media comprises a neutral protease.

[0051] In some examples, the enzymatic media comprises a hyaluronidase.

[0052] In some examples, provided herein is a method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:

- (a) culturing a first population of TILs obtained by digesting in an enzyme media a tumor tissue resected from a subject or patient to produce a tumor digest in a first cell culture medium comprising IL-2 and OKT-3 for about 3-9 days to produce a second population of TILs;
- (b) gene-editing at least a portion of the second population of TILs, to produce a third population of TILs; and
- (c) culturing the third population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.

[0053] In some examples, the step of culturing or initial expansion of the first population of TILs comprises culturing the first population of TILs in the first cell culture medium comprising IL-2 for about 3 days followed by culturing the first population of TILs in a cell culture medium comprising IL-2 and OKT-3 for 2-6 days.

[0054] In some examples, the step of culturing or rapid second expansion of the third population of TILs is performed by culturing the third population of TILs in the second cell culture medium for a first period of about 1-7 days, at the end of the first period the third population of TILs is split into a plurality of subcultures, each of the subcultures is cultured in a third cell culture medium comprising IL-2 for a second period of about 3-7 days, and at the end of the second period the subcultures are combined to provide the expanded number of TILs.

[0055] In some examples, the first period of culturing is about 5 days.

[0056] In some examples, the second period of culturing is about 4 days.

[0057] In some examples, the second period of culturing is about 5 days.

[0058] In some examples, the step of culturing the first population of TILs is performed for about 3 days.

[0059] In some examples, the step of culturing the first population of TILs is performed for about 5 days.

[0060] In some examples, the step of culturing the first population of TILs is performed for about 7 days.

[0061] In some examples, the step of culturing the third population of TILs is performed for about 8 days.

[0062] In some examples, the step of culturing the third population of TILs is performed for about 9 days.

[0063] In some examples, the step of culturing the third population of TILs is performed for about 8-9 days.

[0064] In some examples, the step of culturing the third population of TILs is performed for about 10 days.

[0065] In some examples, the step of culturing the third population of TILs is performed for about 8-10 days.

[0066] In some examples, all steps are completed within a period of about 22 days.

[0067] In some examples, all steps are completed within a period of about 20 days.

[0068] In some examples, all steps are completed within a period of about 22 days.

[0069] In some examples, all steps are completed within a period of about 19-22 days.

[0070] In some examples, all steps are completed within a period of about 19-20 days.

[0071] In some examples, all steps are completed within a period of about 20-22 days.

[0072] In some examples, all steps are completed within a period of about 16-18 days.

[0073] In some examples, in the step of culturing or initial expansion of the first population of TILs in the first culture medium further comprises anti-CD3 and anti-CD28 beads or antibodies.

[0074] In some examples, the anti-CD3 and anti-CD28 beads or antibodies comprise TransAct.

[0075] In some examples, the anti-CD3 and anti-CD28 beads or antibodies comprise TransAct at 1:10, 1:17.5 or 1:100 dilution.

[0076] In some examples, the first culture medium comprises OKT-3 at 300 ng/mL.

[0077] In some examples, the step of culturing or initial expansion of the first population of TILs comprises culturing the first population of TILs in the first cell culture medium comprising IL-2 and anti-CD3 and anti-CD28 beads or antibodies for about 3 days followed by culturing the first population of TILs in a cell culture medium comprising IL-2 and OKT-3 for 2-4 days.

[0078] In some examples, the anti-CD3 and anti-CD28 beads or antibodies comprise TransAct.

[0079] In some examples, the anti-CD3 and anti-CD28 beads or antibodies comprise TransAct at 1:10, 1:17.5 or 1:100 dilution.

[0080] In some examples, the first culture medium comprises OKT-3 at 300 ng/mL.

[0081] In some examples, the expanded number of TILs comprises a therapeutic population of TILs.

[0082] In some examples, the step of gene-editing at least a portion of the second or third population of TILs comprises performing a sterile electroporation step on the second or third population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor.

[0083] In some examples, the step of gene-editing at least a portion of the second or third population of TILs comprises performing a sterile electroporation step on the second or third population of TILs, wherein the sterile electroporation step mediates the transfer of at least two gene editors.

[0084] In some examples, the electroporation step consists of a single electroporation event that mediates the transfer of the at least two gene editors.

[0085] In some examples, in the electroporation step for each of the at least two gene editors is transferred individually by an electroporation event independently of the transfer of any other gene editor.

[0086] In some examples, the electroporation step further comprises a rest period after each electroporation event.

[0087] In some examples, the electroporation step comprises a first electroporation event that mediates the transfer of a first gene editor for modulating expression of a first protein, a first rest period, a second electroporation event that mediates the transfer of a second gene editor for modulating expression of a second protein, and a second rest period, wherein the first and second rest periods are the same or different.

[0088] In some examples, the first and second rest periods comprise incubating the third or fourth population of TILs in the second cell culture medium comprising IL-2 and/or IL-15.

[0089] In some examples, the first and second rest periods comprise incubating the third or fourth population of TILs in the second cell culture medium comprising IL-2 at 300 IU/mL, 1000 IU/mL or 6000 IU/mL.

[0090] In some examples, the first and second rest periods comprise incubating the third or fourth population of TILs in the second cell culture medium comprising IL-15 at 15 ng/mL.

[0091] In some examples, the first and second rest periods comprise incubating the third or fourth population of TILs at about 30-40 °C with about 5% CO₂.

[0092] In some examples, the first and second rest periods comprise incubating the third or fourth population of TILs at about 25, 28, 30, 32, 35 or 37 °C with about 5% CO₂.

[0093] In some examples, the first and second rest periods are independently about 10 hours to 5 days.

[0094] In some examples, the first and second rest periods are independently about 10 hours to 3 days.

[0095] In some examples, the first rest period is about 1 to 3 days.

[0096] In some examples, the first rest period is about 3 days.

[0097] In some examples, the second rest period is about 10 hours to 1 day.

[0098] In some examples, the second rest period is about 12 hours to 24 hours.

[0099] In some examples, the second rest period is about 15 hours to about 18 hours.

[00100] In some examples, the second rest period comprises incubating the third or fourth population of TILs in a cell culture medium comprising IL-2 for about 15 hours to 23 hours at about 30°C.

[00101] In some examples, the second rest period comprises incubating the third or fourth population of TILs in a cell culture medium comprising IL-2 for about for about one hour at 37°C followed by about 15 hours to 23 hours at about 30°C.

[00102] In some examples, the second rest period comprises incubating the third or fourth population of TILs in a cell culture medium comprising IL-2 for about one hour at 37°C followed by about 15 hours to 22 hours at about 30°C.

[00103] In some examples, the first rest period is about 3 days and the second rest period is about 10 to 16 hours.

[00104] In some examples, the electroporation step is preceded by washing the second or third population of TILs in a cytoporation buffer.

[00105] In some examples, the at least one gene editor is a TALE nuclease system for modulating the expression of at least one protein.

[00106] In some examples, the at least one gene editor comprises a TALE nuclease system that modulates expression of PD-1.

[00107] In some examples, the at least one gene editor comprises a TALE nuclease system that modulates expression of CTLA-4.

[00108] In some examples, the at least one gene editor comprises a TALE nuclease system that modulates expression of LAG-3.

[00109] In some examples, the at least one gene editor comprises a TALE nuclease system that modulates expression of CISH.

- [00110]** In some examples, the at least one gene editor comprises a TALE nuclease system that modulates expression of CBL-B.
- [00111]** In some examples, the at least one gene editor comprises a TALE nuclease system that modulates expression of TIGIT.
- [00112]** In some examples, the at least two gene editors comprise a first gene editor comprising a first TALE nuclease system for modulating expression of a first protein and a second gene editor comprising a second TALE nuclease system for modulating expression of a second protein.
- [00113]** In some examples, the first and second TALE nuclease systems modulate expression of PD-1, CTLA-4, LAG-3, CISH, TIGIT and/or CBL-B.
- [00114]** In some examples, the first and second TALE nuclease systems modulate expression of PD-1 and CTLA-4.
- [00115]** In some examples, the first and second TALE nuclease systems modulate expression of PD-1 and LAG-3.
- [00116]** In some examples, the first and second TALE nuclease systems modulate expression of PD-1 and CISH.
- [00117]** In some examples, the first and second TALE nuclease systems modulate expression of PD-1 and CBL-B.
- [00118]** In some examples, the first and second TALE nuclease systems modulate expression of PD-1 and TIGIT.
- [00119]** In some examples, the first and second TALE nuclease systems modulate expression of CTLA-4 and LAG-3.
- [00120]** In some examples, the first and second TALE nuclease systems modulate expression of CTLA-4 and CISH.
- [00121]** In some examples, the first and second TALE nuclease systems modulate expression of CTLA-4 and CBL-B.
- [00122]** In some examples, the first and second TALE nuclease systems modulate expression of LAG-3 and CISH.
- [00123]** In some examples, the first and second TALE nuclease systems modulate expression of LAG-3 and CBL-B.
- [00124]** In some examples, the first and second TALE nuclease systems modulate expression of CISH and CBL-B.

- [00125]** In some examples, the first protein and the second protein are independently selected from the group consisting of PD-1, CTLA-4, LAG-3, CISH, TIGIT and CBL-B, with the proviso that the first protein and the second protein are different.
- [00126]** In some examples, the first protein and the second protein are selected from the group consisting of PD-1 and CTLA-4.
- [00127]** In some examples, the first protein and the second protein are selected from the group consisting of PD-1 and LAG-3.
- [00128]** In some examples, the first protein and the second protein are selected from the group consisting of PD-1 and CISH.
- [00129]** In some examples, the first protein and the second protein are selected from the group consisting of PD-1 and CBL-B.
- [00130]** In some examples, the first protein and the second protein are selected from the group consisting of PD-1 and TIGIT.
- [00131]** In some examples, the first protein and the second protein are selected from the group consisting of CTLA-4 and LAG-3.
- [00132]** In some examples, the first protein and the second protein are selected from the group consisting of CTLA-4 and CISH.
- [00133]** In some examples, the first protein and the second protein are selected from the group consisting of CTLA-4 and CBL-B.
- [00134]** In some examples, the first protein and the second protein are selected from the group consisting of LAG-3 and CISH.
- [00135]** In some examples, the first protein and the second protein are selected from the group consisting of LAG-3 and CBL-B.
- [00136]** In some examples, the first protein and the second protein are selected from the group consisting of CISH and CBL-B.
- [00137]** In some examples, the first protein is PD-1 and the second protein is CTLA-4.
- [00138]** In some examples, the first protein is CTLA-4 and the second protein is PD-1.
- [00139]** In some examples, the first protein is PD-1 and the second protein is LAG-3.
- [00140]** In some examples, the first protein is LAG-3 and the second protein is PD-1.
- [00141]** In some examples, the first protein is PD-1 and the second protein is CISH.
- [00142]** In some examples, the first protein is CISH and the second protein is PD-1.

- [00143] In some examples, the first protein is PD-1 and the second protein is CBL-B.
- [00144] In some examples, the first protein is CBL-B and the second protein is PD-1.
- [00145] In some examples, the first protein is PD-1 and the second protein is TIGIT.
- [00146] In some examples, the first protein is TIGIT and the second protein is PD-1.
- [00147] In some examples, the first protein is CTLA-4 and the second protein is LAG-3.
- [00148] In some examples, the first protein is LAG-3 and the second protein is CTLA-4.
- [00149] In some examples, the first protein is CTLA-4 and the second protein is CISH.
- [00150] In some examples, the first protein is CISH and the second protein is CTLA-4.
- [00151] In some examples, the first protein is CTLA-4 and the second protein is CBL-B.
- [00152] In some examples, the first protein is CBL-B and the second protein is CTLA-4.
- [00153] In some examples, the first protein is LAG-3 and the second protein is CISH.
- [00154] In some examples, the first protein is CISH and the second protein is LAG-3.
- [00155] In some examples, the first protein is LAG-3 and the second protein is CBL-B.
- [00156] In some examples, the first protein is CBL-B and the second protein is LAG-3.
- [00157] In some examples, the first protein is CISH and the second protein is CBL-B.
- [00158] In some examples, the first protein is CBL-B and the second protein is CISH.
- [00159] In some examples, the first protein or the second protein is PD-1.
- [00160] In some examples, the first protein or the second protein is CTLA-4.
- [00161] In some examples, the first protein or the second protein is LAG-3.
- [00162] In some examples, the first protein or the second protein is CISH.
- [00163] In some examples, the first protein or the second protein is CBL-B.
- [00164] In some examples, the first protein or the second protein is TIGIT.
- [00165] In some examples, the first gene editor downregulates expression of the first protein and the second gene editor downregulates expression of the second protein.
- [00166] In some examples, the antigen presenting cells (APCs) are PBMCs.
- [00167] In some examples, the PBMCs are irradiated and allogeneic.
- [00168] In some examples, the antigen-presenting cells are artificial antigen-presenting cells.
- [00169] In some examples, the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL.

[00170] In some examples, the first cell culture medium and/or the second cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist.

[00171] In some examples, the tumor tissue is processed into multiple tumor fragments.

[00172] In some examples, the tumor fragments are added into the closed system.

[00173] In some examples, 150 or fewer of the fragments, 100 or fewer of the fragments, or 50 or fewer of the fragments are added into the closed system.¹⁶⁷ A gene-edited population of tumor infiltrating lymphocytes (TILs) comprising an expanded population of TILs wherein the expression of at least one protein is modulated by a gene editor transferred into at least a portion of the expanded population of TILs.

[00174] In some examples, the gene editor is a TALE nuclease system for modulating the expression of the at least one protein.

[00175] In some examples, the at least one protein is PD-1.

[00176] In some examples, the at least one protein is CTLA-4.

[00177] In some examples, the at least one protein is LAG-3.

[00178] In some examples, the at least one protein is CISH.

[00179] In some examples, the at least one protein is CBL-B.

[00180] In some examples, the at least one protein is TIGIT.

[00181] In some examples, the expression of at least two proteins is modulated by at least two gene editors transferred into at least a portion of the expanded population of TILs, wherein the at least two gene editors comprise a first gene editor comprising a first TALE nuclease system for modulating expression of a first protein and a second gene editor comprising a second TALE nuclease system for modulating expression of a second protein.

[00182] In some examples, the first and second proteins are independently selected from the group consisting of PD-1, CTLA-4, LAG-3, CISH, TIGIT and CBL-B, with the proviso that the first protein and the second protein are different.

[00183] In some examples, the first and second proteins are selected from the group consisting of PD-1 and CTLA-4.

[00184] In some examples, the first and second proteins are selected from the group consisting of PD-1 and LAG-3.

[00185] In some examples, the first and second proteins are selected from the group consisting of PD-1 and CISH.

[00186] In some examples, the first and second proteins are selected from the group consisting of PD-1 and CBL-B.

[00187] In some examples, the first and second proteins are selected from the group consisting of PD-1 and TIGIT.

[00188] In some examples, the first and second proteins are selected from the group consisting of CTLA-4 and LAG-3.

[00189] In some examples, the first and second proteins are selected from the group consisting of CTLA-4 and CISH.

[00190] In some examples, the first and second proteins are selected from the group consisting of CTLA-4 and CBL-B.

[00191] In some examples, the first and second TALE proteins are selected from the group consisting of LAG-3 and CISH.

[00192] In some examples, the first and second proteins are selected from the group consisting of LAG-3 and CBL-B.

[00193] In some examples, the first and second proteins are selected from the group consisting of CISH and CBL-B.

[00194] In some examples, the gene-edited population of TILs disclosed herein is manufactured by a method disclosed herein.

[00195] In some examples, provided herein is a pharmaceutical composition comprising the gene edited population of TILs disclosed herein and a pharmaceutically acceptable carrier.

[00196] In some examples, provided herein is a method for treating a subject with cancer, the method comprising administering a therapeutically effective dose of the gene edited population of TILs disclosed herein.

[00197] In some examples, the cancer is selected from the group consisting of melanoma, metastatic melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), metastatic 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)), renal cancer, and renal cell carcinoma.

[00198] In some examples, provided herein is a method for treating a subject with cancer, the method comprising administering expanded tumor infiltrating lymphocytes (TILs) comprising:

(a) obtaining a first population of TILs from a tumor resected from a patient by processing a

tumor sample obtained from the patient into multiple tumor fragments;

(b) adding the tumor fragments into a closed system and performing a first expansion by culturing the first population of TILs in a first 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 3-8 days to obtain the second population of TILs;

(c) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 and anti-CD28 agonist beads or antibodies, for 1-6 days, to produce a third population of TILs;

(e) performing a sterile electroporation step on the third population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;

(f) resting the third population of TILs for about 1 day;

(g) performing a second expansion by culturing the third population of TILs in a second cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs), to produce a fourth population of TILs, wherein the second expansion is performed for about 5-15 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, wherein the fourth population of TILs is a therapeutic population of TILs;

(h) harvesting the therapeutic population of TILs obtained from step (e) to provide a harvested TIL population, wherein one or more of steps (a) to (h) are performed in a closed, sterile system;

(i) transferring the harvested TIL population to an infusion bag, wherein the transfer from step (h) to (i) occurs without opening the system;

(j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based cryopreservation medium; and

(k) administering a therapeutically effective dosage of the harvested TIL population from the infusion bag to the patient;

wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector Nuclease (TALEN) system for inhibiting the expression of PD-1, CTLA-4, LAG-3, CISH, TIGIT and/or CBL-B.

[00199] In some examples, the electroporation step comprises the delivery of a TALEN system for inhibiting the expression of PD-1.

[00200] In some examples, the electroporation step comprises the delivery of a TALEN system for inhibiting the expression of CTLA-4.

[00201] In some examples, the electroporation step comprises the delivery of a TALEN system for inhibiting the expression of LAG-3.

[00202] In some examples, the electroporation step comprises the delivery of a TALEN system for inhibiting the expression of CISH.

[00203] In some examples, the electroporation step comprises the delivery of a TALEN system for inhibiting the expression of CBL-B.

[00204] In some examples, the electroporation step comprises the delivery of a TALEN system for inhibiting the expression of TIGIT.

[00205] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of PD-1 and CTLA-4.

[00206] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of PD-1 and LAG-3.

[00207] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of PD-1 and CISH.

[00208] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of PD-1 and CBL-B.

[00209] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of PD-1 and TIGIT.

[00210] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CTLA-4 and LAG-3.

[00211] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CTLA-4 and CISH.

[00212] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CTLA-4 and CBL-B.

[00213] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CTLA-4 and TIGIT.

[00214] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of LAG-3 and CISH.

[00215] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of LAG-3 and CBL-B.

[00216] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of LAG-3 and TIGIT.

[00217] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CISH and CBL-B.

[00218] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CISH and TIGIT.

[00219] In some examples, the electroporation step comprises the delivery of TALEN systems for inhibiting the expression of CBL-B and TIGIT.

[00220] In some examples, the therapeutically effective dosage of TILs is from about 1×10^9 to about 1×10^{11} TILs.

[00221] In some examples, prior to administering a therapeutically effective dosage of the harvested TIL population in step (k), a non-myeloablative lymphodepletion regimen has been administered to the patient.

[00222] In some examples, the method further comprises the step of treating the patient with a high-dose IL-2 regimen starting on the day after administration of the therapeutically effective dosage of the harvested TIL population to the patient in step (k).

[00223] In some examples, the cancer is selected from the group consisting of melanoma, metastatic melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), metastatic 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)), renal cancer, and renal cell carcinoma.

[00224] In some examples, the cancer is melanoma.

[00225] In some examples, the cancer is metastatic melanoma.

[00226] In some examples, the cancer is NSCLC.

[00227] In some examples, the cancer is metastatic NSCLC.

[00228] In some examples, the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.

CLAIMS

1. A method for preparing expanded tumor infiltrating lymphocytes (TILs) comprising:
 - (a) culturing a first population of TILs obtained and/or received from a tumor tissue resected from a subject or patient in a first cell culture medium comprising IL-2 for about 3-9 days to produce a second population of TILs;
 - (b) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 agonist and anti-CD28 agonist beads or antibodies, for 1-7 days, to produce a third population of TILs;
 - (c) gene-editing at least a portion of the third population of TILs, to produce a fourth population of TILs; and
 - (d) culturing the fourth population of TILs in a second cell culture medium comprising antigen presenting cells (APCs), OKT-3, and IL-2 for about 5-15 days, to produce an expanded number of TILs.
2. The method of claim 1, wherein
 - (i) the step of culturing the fourth population of TILs is performed by culturing the fourth population of TILs in the second cell culture medium for a first period of about 1-7 days, at the end of the first period the fourth population of TILs is split into a plurality of subcultures, each of the subcultures is cultured in a third cell culture medium comprising IL-2 for a second period of about 3-7 days, and at the end of the second period the subcultures are combined to provide the expanded number of TILs or the therapeutic population of TILs, wherein optionally the first period of culturing is about 5 days, wherein further optionally the second period of culturing is about 4 days or 5 days;
 - (ii) the step of activating the second population of TILs is performed using anti-CD3 agonist beads or antibodies,wherein optionally the step of activating the second population of TILs is performed using OKT-3,

wherein further optionally the step of activating the second population of TILs is performed using OKT-3 at 300 ng/mL;

(iii) the step of activating the second population of TILs is performed using anti-CD3 agonist and anti-CD28 agonist beads or antibodies,

wherein optionally the step of activating the second population of TILs is performed using TransAct,

wherein further optionally the step of activating the second population of TILs is performed using TransAct at 1:10, 1:17.5 or 1:100 dilution;

(iv) the step of activating the second population of TILs is performed for about 2 days, about 3 days, about 4 days or about 5 days;

(v) the step of culturing the first population of TILs is performed for about 3 days, about 5 days, or about 7 days;

(vi) the step of culturing the fourth population of TILs is performed for about 8 days, about 9 days, 8-9 days; about 10 days or 8-10 days; or

(vii) all steps are completed within a period of about 22 days, 19-22 days, 19-20 days, or 20-22 days.

3. The method of claim 1 or 2, wherein the expanded number of TILs comprises a therapeutic population of TILs.
4. The method of any one of claims 1-3, wherein the step of gene-editing at least a portion of the second or third population of TILs comprises performing a sterile electroporation step on the second or third population of TILs, wherein the sterile electroporation step mediates the transfer of one gene editor or the transfer of at least two gene editors, wherein optionally:
 - (i) the electroporation step consists of a single electroporation event that mediates the transfer of the one gene editor or the transfer of the at least two gene editors;
 - (ii) the electroporation step for each of the at least two gene editors is transferred individually by an electroporation event independently of the transfer of any other gene editor;
 - (iii) the electroporation step further comprises a rest period after each electroporation event;
 - (iv) the electroporation step comprises a first electroporation event that mediates the transfer of a first gene editor for modulating expression of a first protein, a first rest period,

a second electroporation event that mediates the transfer of a second gene editor for modulating expression of a second protein, and a second rest period, wherein the first and second rest periods are the same or different;

(v) the first and second rest periods comprise incubating the third or fourth population of TILs in the second cell culture medium comprising IL-2 and/or IL-15;

(vi) the first and second rest periods comprise incubating the third or fourth population of TILs in the second cell culture medium comprising IL-2 at 300 IU/mL, 1000 IU/mL or 6000 IU/mL; or

(vii) the first and second rest periods comprise incubating the third or fourth population of TILs in the second cell culture medium comprising IL-15 at 15 ng/mL.

5. The method of claim 4, wherein

(i) the first and second rest periods comprise incubating the third or fourth population of TILs at 30-40 °C with about 5% CO₂,

wherein optionally the first and second rest periods comprise incubating the third or fourth population of TILs at about 25, 28, 30, 32, 35 or 37 °C with about 5% CO₂;

(ii) the first and second rest periods are independently 10 hours to 5 days; or

(iii) the first and second rest periods are independently 10 hours to 3 days;

wherein optionally,

(a) the first rest period is 1 to 3 days;

(b) the first rest period is about 3 days;

(c) the second rest period is 10 hours to 1 day;

(d) the second rest period is 12 hours to 24 hours;

(e) the second rest period is 15 hours to 18 hours;

(f) the second rest period comprises incubating the third or fourth population of TILs in a cell culture medium comprising IL-2 for 15 hours to 23 hours at about 30°C;

(g) the second rest period comprises incubating the third or fourth population of TILs in a cell culture medium comprising IL-2 for about one hour at 37°C followed by 15 hours to 23 hours at about 30°C;

(h) the second rest period comprises incubating the third or fourth population of TILs in a cell culture medium comprising IL-2 for about one hour at 37°C followed by 15 hours to 22 hours at about 30°C; or

(i) wherein the first rest period is about 3 days and the second rest period is 10 to 16 hours.

6. The method of claim 4 or 5, wherein the electroporation step is preceded by washing the second or third population of TILs in a cytoporation buffer.
7. The method of any one of claims 4-6, wherein the one gene editor is a TALE nuclease system for modulating the expression of one protein, wherein optionally:
 - (i) the one gene editor comprises a TALE nuclease system that modulates expression of PD-1;
 - (ii) the one gene editor comprises a TALE nuclease system that modulates expression of CTLA-4;
 - (iii) the one gene editor comprises a TALE nuclease system that modulates expression of LAG-3;
 - (iv) the one gene editor comprises a TALE nuclease system that modulates expression of CISH;
 - (v) the one gene editor comprises a TALE nuclease system that modulates expression of CBL-B; or
 - (vi) the one gene editor comprises a TALE nuclease system that modulates expression of TIGIT.
8. The method of any one of claims 4-6, wherein the at least two gene editors comprise a first gene editor comprising a first TALE nuclease system for modulating expression of a first protein and a second gene editor comprising a second TALE nuclease system for modulating expression of a second protein, wherein optionally:

- (i) the first and second TALE nuclease systems modulate expression of PD-1, CTLA-4, LAG-3, CISH, TIGIT and/or CBL-B;
 - (ii) the first and second TALE nuclease systems modulate expression of PD-1 and CTLA-4;
 - (iii) the first and second TALE nuclease systems modulate expression of PD-1 and LAG-3;
 - (iv) the first and second TALE nuclease systems modulate expression of PD-1 and CISH;
 - (v) the first and second TALE nuclease systems modulate expression of PD-1 and CBL-B;
 - (vi) the first and second TALE nuclease systems modulate expression of PD-1 and TIGIT;
 - (vii) the first and second TALE nuclease systems modulate expression of CTLA-4 and LAG-3;
 - (viii) the first and second TALE nuclease systems modulate expression of CTLA-4 and CISH;
 - (ix) the first and second TALE nuclease systems modulate expression of CTLA-4 and CBL-B;
 - (x) the first and second TALE nuclease systems modulate expression of LAG-3 and CISH;
 - (xi) the first and second TALE nuclease systems modulate expression of LAG-3 and CBL-B; or
 - (xii) the first and second TALE nuclease systems modulate expression of CISH and CBL-B.
9. The method of claim 8, wherein the first protein and the second protein are independently selected from the group consisting of PD-1, CTLA-4, LAG-3, CISH, TIGIT and CBL-B, with the proviso that the first protein and the second protein are different,
- wherein optionally the first protein and the second protein are selected from:
- (i) the group consisting of PD-1 and CTLA-4;
 - (ii) the group consisting of PD-1 and LAG-3;
 - (iii) the group consisting of PD-1 and CISH;
 - (iv) the group consisting of PD-1 and CBL-B;
 - (v) the group consisting of PD-1 and TIGIT;
 - (vi) the group consisting of CTLA-4 and LAG-3;
 - (vii) the group consisting of CTLA-4 and CISH;

(viii) the group consisting of CTLA-4 and CBL-B;

(ix) the group consisting of LAG-3 and CISH;

(x) the group consisting of LAG-3 and CBL-B; or

(xi) the group consisting of CISH and CBL-B;

wherein further optionally:

(a) the first protein is PD-1 and the second protein is CTLA-4;

(b) the first protein is CTLA-4 and the second protein is PD-1;

(c) the first protein is PD-1 and the second protein is LAG-3;

(d) the first protein is LAG-3 and the second protein is PD-1;

(e) the first protein is PD-1 and the second protein is CISH;

(f) the first protein is CISH and the second protein is PD-1;

(g) the first protein is PD-1 and the second protein is CBL-B;

(h) the first protein is CBL-B and the second protein is PD-1;

(i) the first protein is PD-1 and the second protein is TIGIT;

(j) the first protein is TIGIT and the second protein is PD-1;

(k) the first protein is CTLA-4 and the second protein is LAG-3;

(l) wherein the first protein is LAG-3 and the second protein is CTLA-4;

(m) the first protein is CTLA-4 and the second protein is CISH;

(n) the first protein is CISH and the second protein is CTLA-4;

(o) the first protein is CTLA-4 and the second protein is CBL-B;

(p) the first protein is CBL-B and the second protein is CTLA-4;

(q) the first protein is LAG-3 and the second protein is CISH;

(r) the first protein is CISH and the second protein is LAG-3;

- (s) the first protein is LAG-3 and the second protein is CBL-B;
 - (t) the first protein is CBL-B and the second protein is LAG-3;
 - (u) the first protein is CISH and the second protein is CBL-B;
 - (v) the first protein is CBL-B and the second protein is CISH; or
 - (w) the first protein or the second protein is: PD-1 CTLA-4, LAG-3, CISH, CBL-B, or TIGIT.
10. The method of any of claims 4-6 or 8-9, wherein the first gene editor downregulates expression of the first protein and the second gene editor downregulates expression of the second protein.
 11. The method of any one of claims 1-10, wherein the antigen presenting cells (APCs) are
 - (a) PBMCs; wherein optionally the PBMCs are irradiated and allogeneic; or
 - (b) artificial antigen-presenting cells.
 12. The method according to any one of claims 1-11, wherein
 - (i) the IL-2 concentration is about 10,000 IU/mL to about 5,000 IU/mL;
 - (ii) the first cell culture medium and/or the second cell culture medium further comprises a 4-1BB agonist and/or an OX40 agonist; or
 - (iii) the tumor tissue is processed into multiple tumor fragments,
 wherein optionally the tumor fragments are added into the closed system,

 wherein further optionally 150 or fewer of the fragments, 100 or fewer of the fragments, or 50 or fewer of the fragments are added into the closed system.
 13. A gene-edited population of tumor infiltrating lymphocytes (TILs) comprising an expanded population of TILs wherein the expression of at least one protein is modulated by a gene editor transferred into at least a portion of the expanded population of TILs.
 14. The gene-edited population of TILs of claim 13, wherein the gene editor is a TALE nuclease system for modulating the expression of the one protein, wherein optionally the one protein is:
 - (a) PD-1;

- (b) CTLA-4;
 - (c) LAG-3;
 - (d) CISH;
 - (e) CBL-B; or
 - (f) TIGIT.
15. The gene-edited population of TILs of claim 13, wherein the expression of at least two proteins is modulated by at least two gene editors transferred into at least a portion of the expanded population of TILs, wherein the at least two gene editors comprise a first gene editor comprising a first TALE nuclease system for modulating expression of a first protein and a second gene editor comprising a second TALE nuclease system for modulating expression of a second protein, wherein optionally the first and second proteins:
- (i) are independently selected from the group consisting of PD-1, CTLA-4, LAG-3, CISH, TIGIT and CBL-B, with the proviso that the first protein and the second protein are different;
 - (ii) are selected from the group consisting of PD-1 and CTLA-4;
 - (iii) are selected from the group consisting of PD-1 and LAG-3;
 - (iv) are selected from the group consisting of PD-1 and CISH;
 - (v) are selected from the group consisting of PD-1 and CBL-B;
 - (vi) are selected from the group consisting of PD-1 and TIGIT;
 - (vii) are selected from the group consisting of CTLA-4 and LAG-3;
 - (viii) are selected from the group consisting of CTLA-4 and CISH;
 - (ix) are selected from the group consisting of CTLA-4 and CBL-B;
 - (x) are selected from the group consisting of LAG-3 and CISH;
 - (xi) are selected from the group consisting of LAG-3 and CBL-B; or
 - (xii) are selected from the group consisting of CISH and CBL-B.
16. A therapeutically effective dose of the gene edited population of TILs of any one of claims 13-15 for use in therapy.

17. Use of a therapeutically effective dose of the gene edited population of tumor infiltrating lymphocytes (TILs) of any one of claims 13-15 in the manufacture of a medicament for treating cancer, wherein the cancer is selected from the group consisting of melanoma, metastatic melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), metastatic 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)), renal cancer, and renal cell carcinoma.
18. Use of an expanded tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating cancer, the use comprises:
 - (a) obtaining a first population of TILs from a patient by processing a tumor sample obtained from the patient into multiple tumor fragments;
 - (b) performing a first expansion by culturing the first population of TILs in a first 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 3-8 days to obtain the second population of TILs;
 - (c) activating the second population of TILs using anti-CD3 agonist beads or antibodies, or anti-CD3 and anti-CD28 agonist beads or antibodies, for 1-6 days, to produce a third population of TILs;
 - (e) performing a sterile electroporation step on the third population of TILs, wherein the sterile electroporation step mediates the transfer of at least one gene editor;
 - (f) resting the third population of TILs for about 1 day;
 - (g) performing a second expansion by culturing the third population of TILs in a second cell culture medium comprising IL-2, OKT-3, and antigen presenting cells (APCs), to produce a fourth population of TILs, wherein the second expansion is performed for about 5-15 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, wherein the fourth population of TILs is a therapeutic population of TILs;
 - (h) harvesting the therapeutic population of TILs obtained from step (e) to provide a harvested TIL population;
 - (i) transferring the harvested TIL population to an infusion bag;
 - (j) cryopreserving the harvested TIL population using a dimethylsulfoxide-based

cryopreservation medium; and

(k) a therapeutically effective dosage of the harvested TIL population from the infusion bag to be administered to the patient;

wherein the electroporation step comprises the delivery of a Transcription Activator-Like Effector Nuclease (TALEN) system for inhibiting the expression of PD-1, CTLA-4, LAG-3, CISH, TIGIT and/or CBL-B.

19. The use of claim 18, wherein the electroporation step comprises the delivery of:

- (i) a TALEN system for inhibiting the expression of PD-1;
- (ii) a TALEN system for inhibiting the expression of CTLA-4;
- (iii) a TALEN system for inhibiting the expression of LAG-3;
- (iv) a TALEN system for inhibiting the expression of CISH;
- (v) a TALEN system for inhibiting the expression of CBL-B;
- (vi) a TALEN system for inhibiting the expression of TIGIT;
- (vii) TALEN systems for inhibiting the expression of PD-1 and CTLA-4;
- (viii) TALEN systems for inhibiting the expression of PD-1 and LAG-3;
- (ix) TALEN systems for inhibiting the expression of PD-1 and CISH;
- (x) TALEN systems for inhibiting the expression of PD-1 and CBL-B;
- (xi) TALEN systems for inhibiting the expression of PD-1 and TIGIT;
- (xii) TALEN systems for inhibiting the expression of CTLA-4 and LAG-3;
- (xiii) TALEN systems for inhibiting the expression of CTLA-4 and CISH;
- (xiv) TALEN systems for inhibiting the expression of CTLA-4 and CBL-B;
- (xv) TALEN systems for inhibiting the expression of CTLA-4 and TIGIT;
- (xvi) TALEN systems for inhibiting the expression of LAG-3 and CISH;
- (xvii) TALEN systems for inhibiting the expression of LAG-3 and CBL-B;

- (xviii) TALEN systems for inhibiting the expression of LAG-3 and TIGIT;
 - (xix) TALEN systems for inhibiting the expression of CISH and CBL-B;
 - (xx) TALEN systems for inhibiting the expression of CISH and TIGIT; or
 - (xxi) TALEN systems for inhibiting the expression of CBL-B and TIGIT.
20. The use according to claims 18 or 19, wherein the therapeutically effective dosage of TILs is from 1×10^9 to 1×10^{11} TILs, wherein optionally:
- (a) prior to a therapeutically effective dosage of the harvested TIL population in step (k) is to be administered, a non-myeloablative lymphodepletion regimen has been administered to the patient;
 - (b) the use further comprises the step of the patient is to be treated with a high-dose IL-2 regimen starting on the day after administration of the therapeutically effective dosage of the harvested TIL population to the patient in step (k);
 - (c) the cancer is selected from the group consisting of melanoma, metastatic melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), metastatic 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)), renal cancer, and renal cell carcinoma;
 - (d) the cancer is melanoma; wherein optionally the cancer is metastatic melanoma;
 - (e) the cancer is NSCLC; wherein optionally the cancer is metastatic NSCLC; or
 - (f) the gene-editing causes expression of one or more immune checkpoint genes to be silenced or reduced in at least a portion of the therapeutic population of TILs.