CLAIMS

~~What is claimed:~~

1. A method of producing an expanded population of tumor infiltrating lymphocytes (TILs), the method comprising culturing the disaggregated tumor sample in a culture medium comprising (a) feeder cells or an agonist of a T cell costimulatory molecule, (b) a T cell receptor (TCR) agonist, and (c) interleukin (IL)-15, thereby producing an expanded population of TILs.

2. The method of claim 1, wherein the culture medium comprises the IL-15 at a concentration of greater than 100 ng/ml or ~~.~~

~~3. The method of claim 1 or 2, wherein the culture medium comprises the IL-15 at a concentration of~~ less than 1000 ng/ml.

~~4~~3. The method of ~~any one of the preceding claims~~claim 1 or 2, wherein the culture medium does not comprise IL-2 and/or ~~.~~

~~5. The method of any one of the preceding claims, wherein the culture medium does not comprise~~ IL-21.

~~6~~4. The method of any one of the preceding claims, wherein the culture medium further comprises IL-7, optionally at a concentration of 10 U/ml to 7,000 U/ml.

~~7~~5. The method of any one of the preceding claims, wherein:

the TCR agonist is selected from a CD3 agonist, OKT3, and UCHT1;~~.~~

~~8. The method of claim 7, wherein~~

the CD3 agonist is an anti-CD3 antibody, optionally a humanized anti-CD3 antibody, or a soluble monospecific complex comprising two anti-CD3 antibodies linked together; and/or~~.~~

~~9. The method of any one of the preceding claims, wherein~~

the agonist of the T cell costimulatory molecule is selected from: a CD28 agonist, a CD137 agonist, a CD2 agonist, and combinations thereof.

6~~10~~. The method of claim ~~9~~ 5, wherein:

the CD28 agonist comprises a soluble monospecific complex comprising two anti-CD28 antibodies linked together; or

the CD2 agonist comprises a soluble monospecific complex comprising two anti-CD2 antibodies linked together.

~~11~~7. The method of any one of the preceding claims, wherein the TCR agonist and/or the agonist of a T cell costimulatory molecule are linked to a nanomatrix comprising a colloidal suspension of matrices of polymer chains, wherein each matrix is 1 to 500 nm in length in its largest dimension.

~~12~~8. The method of any one of the preceding claims, wherein:

(a) the disaggregated tumor sample comprises tumor fragments optionally generated by a dissection method, that are 0.5 to 4 mm^3 in size; or

(b) the disaggregated tumor sample comprises tumor fragments optionally generated by a mechanical method, that are 25 to 30 mm^3 in size,

optionally wherein the tumor fragments of (a) and (b) comprise digested tumor fragments.

~~13~~9. The method of any one of the preceding claims, wherein cells of the expanded TIL population are genetically modified, optionally epigenetically modified.

1~~14~~0. The method of any one of the preceding claims, further comprising genetically modifying cells of the expanded TIL population using a gene-regulating system, optionally selected from a gene-regulating system comprising RNA interference molecules, transcription activator-like effector nucleases, zinc finger nucleases, and RNA-guided nucleases.

~~15~~11. The method of claim ~~14~~10, wherein gene-regulating system comprises a Cas enzyme, optionally a Cas9 enzyme, and a guide RNA.

~~16~~12. The method of any one of claims ~~13-15~~9-11, wherein cells of the TIL population comprise a modification, optionally an insertion, deletion, indel, or substitution, at one or more gene(s) selected from: *ANKRD11, BCL2L11, BCL3, BCOR, CALM2, CBLB, CHIC2, CTLA4, DHODH, E2F8, EGR2, FLI1, FOXP3, GATA3, GNAS, HAVCR2, IKZF1, IKZF2, IKZF3, LAG3, MAP4K, NFKBIA, NR4A3, NRP1, PBRM1, PCBP1, PDCD1, PELI1, PIK3CD, PPP2R2D, PTPN1, PTPN2, PTPN22, PTPN6, RBM39, RC3H1, SEMA7A, SERPINA3, SETD5, SH2B3, SH2D1A, SMAD2, SOCS1, TANK, TGFBR1, TGFBR2, TIGIT, TNFAIP3, TNIP1, TRAF6, UMPS, WDR6* and *ZC3H12A*, optionally wherein the modification results in reduction or inhibition of expression of the one or more gene(s) and/or function of one or more protein(s) encoded by the one or more gene(s), optionally ~~.~~

~~17. The method of claim 16,~~ wherein cells of the TIL population comprise a modification, optionally an insertion, deletion, indel, or substitution, at the *SOCS1* gene and the *ZC3H12A* gene.

~~18~~13. The method of any one of the preceding claims, wherein at least a portion of the culture medium is changed and/or supplemented with IL-15 during the culturing and/or ~~.~~

~~19. The method of any one of the preceding claims,~~ wherein the culturing occurs over a period of 9-25 days, 9-21 days, or 9-14 days.

~~20~~14. The method of any one of the preceding claims, wherein at least 10% or at least 15% of the expanded population of TILs have a central memory T cell phenotype.

~~21~~15. A method of producing an expanded population of tumor infiltrating lymphocytes (TILs), the method comprising:

culturing a disaggregated tumor sample in a first medium comprising a T cell-stimulating cytokine to produce a population of TILs; and

culturing cells of the population of TILs in a second medium comprising feeder cells or an agonist of a T cell costimulatory molecule, a T cell receptor (TCR) agonist, and interleukin (IL)-15, thereby producing an expanded population of TILs, optionally wherein the method~~.~~

~~22. The method of claim 21,~~ further ~~comprising~~ comprises modifying cells of the population of TILs from the first medium using a gene-regulating system to produce a subpopulation of modified TILs, wherein the population of TILs cultured in the second medium includes the subpopulation of modified TILs, and optionally~~.~~

~~23. The method of claim 21 or 22,~~ wherein the first and/or second medium does not comprise —IL-2.

~~24~~16. A method for expanding a population of tumor infiltrating lymphocytes (TILs) comprising: culturing the population of TILs in a culture medium comprising (a) IL-15 and (b) a nanomatrix comprising a colloidal suspension of matrices of polymer chains, wherein the matrices are attached to TCR agonists and agonists of a T cell costimulatory molecule, each matrix is 1 to 500 nm in length in its largest dimension, and optionally the method does not comprise the use of feeder cells during expansion of the population of TILs.

~~25~~17. A method for expanding a population of tumor infiltrating lymphocytes (TILs) comprising: culturing the population of TILs in a culture medium comprising (a) IL-15, (b) a first soluble monospecific complex comprising an anti-CD3 antibody or fragment thereof, (c) a second soluble monospecific complex comprising an anti-CD28 antibody or fragment thereof, and (d) a third soluble monospecific complex comprising an anti-CD2 antibody or fragment thereof, wherein each of the soluble monospecific complexes comprises two antibodies, or fragments thereof, linked together, and each antibody, or fragments thereof, of each of the soluble monospecific complexes specifically binds to the same antigen on the population of TILs.

~~26~~18. A composition comprising an expanded population of tumor infiltrating lymphocytes (TILs) produced by the method of any one of the preceding claims.

~~27~~19. A composition comprising a disaggregated tumor sample and/or tumor infiltrating lymphocytes (TILs) in a culture medium comprising (a) feeder cells or an agonist of a T cell costimulatory molecule, (b) a T cell receptor (TCR) agonist, and (c) interleukin (IL)-15, optionally at a concentration of greater than 100 ng/ml and less than 1000 ng/ml, optionally ~~.~~

28. ~~The composition of claim 27,~~ wherein the composition does not comprise IL-2.