

CLAIMS

~~What is claimed is:~~

1. A method of expanding a population of tumor infiltrating lymphocytes (TILs) in a disaggregated tumor sample, the method comprising culturing the disaggregated tumor sample in a medium, wherein the TILs are contacted with a T cell receptor (TCR) agonist, a CD28 agonist, and a T cell-stimulating cytokine.

2. The method of claim 1, wherein the medium is supplemented with the T cell-stimulating cytokine at a time interval selected from the group consisting of 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days; and/or

wherein the final concentration of the T cell-stimulating cytokine is 10 U/ml to 7,000 U/ml; and/or

wherein the T cell-stimulating cytokine is IL-2.

~~3. The method of claim 1 or 2, wherein the final concentration of the T cell-stimulating cytokine is 10 U/ml to 7,000 U/ml.~~

~~4. The method of any one of the preceding claims, wherein the T cell-stimulating cytokine is IL-2.~~

~~5. The method of any one of the preceding claims, wherein the medium is changed at a time interval selected from the group consisting of 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days.~~

~~6. The method of any one of the preceding claims, wherein the components of the medium are maintained.~~

~~7. The method of any one of the preceding claims, wherein 30% to 99% of the medium is changed at a time interval selected from the group consisting of 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days.~~

~~8. The method of any one of the preceding claims, wherein the tumor sample is from a subject who had previously submitted a tumor sample for TIL expansion, wherein the previous TIL expansion comprises a pre-REP step and wherein the number of TILs isolated from the pre-REP step was less than 1000 TILs.~~

~~9. The method of any of one of the preceding claims, wherein the tumor sample is from a subject who had previously submitted a tumor sample for TIL expansion, wherein the previous TIL expansion comprises a pre-REP step and wherein the fold expansion of TILs isolated from the pre-REP step was less than 5 fold.~~

~~103.~~ The method of any one of the preceding claims,

wherein the disaggregated tumor sample comprises tumor fragments that are 0.5 to 4 mm³ in size; and/or

wherein the disaggregated tumor sample comprises digested tumor fragments.

~~11. The method of any one of the preceding claims, wherein the disaggregated tumor sample comprises digested tumor fragments.~~

~~124.~~ The method of any one of the preceding claims, wherein the medium comprises feeder cells.

~~135.~~ The method of claim ~~124~~, wherein the feeder cells:

are peripheral blood mononuclear cells or antigen presenting cells, optionally K562 cells; and/or

express the TCR agonist, the CD28 agonist and/or a 4-1BB ligand, optionally on the surface of the feeder cells; and/or

are genetically modified to express the TCR agonist, the CD28 agonist and/or the 4-1BB ligand; and/or

are genetically modified to express the T cell-stimulating cytokine, optionally IL-2.

~~14. The method of claim 12 or 13, wherein the feeder cells express the TCR agonist, the CD28 agonist and/or a 4-1BB ligand.~~

~~15. The method of claim 14, wherein the TCR agonist, the CD28 agonist and/or the 4-1BB ligand are expressed on the surface of the feeder cells.~~

~~16. The method of claim 14 or 15, wherein the feeder cells are genetically modified to express the TCR agonist, the CD28 agonist and/or the 4-1BB ligand.~~

~~176.~~ The method of claim ~~165~~, wherein:

the TCR agonist is a CD3 agonist; and/or

the CD3 agonist is OKT3; and/or

- the CD28 agonist is CD86.
- ~~18. The method of claim 17, wherein the CD3 agonist is OKT3.~~
- ~~19. The method of claim 16, wherein the CD28 agonist is CD86.~~
- ~~20. The method of any one of claims 12-19, wherein the feeder cells are antigen presenting cells.~~
- ~~21. The method of claim 20, wherein the antigen presenting cells comprise K562 cells.~~
- ~~22. The method of any one of claims 12 to 21, wherein the feeder cells are genetically modified to express the T cell stimulating cytokine.~~
- ~~23. The method of claim 22, wherein the T cell stimulating cytokine is IL-2.~~
- ~~24. The method of any one of claims 1 to 23, wherein the medium does not comprise feeder cells.~~
- ~~25. The method of any of the preceding claims, wherein the CD28 agonist is soluble in the medium.~~
- ~~26. The method of any one of the preceding claims, wherein the TCR agonist is a CD3 agonist.~~
- ~~27. The method of any one of the preceding claims, wherein the TCR agonist and/or the CD28 agonist are linked to a nanomatrix comprising a colloidal suspension of matrices of polymer chains, wherein each nanomatrix is 1 to 500 nm in length in its largest dimension, optionally wherein the TCR agonist and the CD28 agonist are attached to the same polymer chains or to different polymer chains.~~
- ~~28. The method of claim 27, wherein the TCR agonist and the CD28 agonist are attached to the same polymer chains.~~
- ~~29. The method of claim 27, wherein the TCR agonist and the CD28 agonist are attached to different polymer chains.~~
- ~~30. The method of any one of claims 27 to 29, wherein the TCR agonist is attached to the nanomatrix at 25 µg per mg of nanomatrix.~~
- ~~31. The method of any one of claims 1 to 30, wherein:~~
- the TCR agonist comprises a soluble monospecific complex comprising two anti-CD3 antibodies linked together; and/or

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

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

~~32. The method of any one of claims 1 to 26, and 31, wherein the CD28 agonist comprises a soluble monospecific complex comprising two anti-CD28 antibodies linked together.~~

~~33. The method of any one of claims 1 to 26, 31, and 32, wherein the medium comprises a CD2 agonist.~~

~~34. The method of claim 33, wherein the CD2 agonist comprises a soluble monospecific complex comprising two anti-CD2 antibodies linked together.~~

3510. A method for expanding a population of tumor infiltrating lymphocytes (TILs) comprising contacting the population of TILs with a nanomatrix comprising a colloidal suspension of matrices of polymer chains, wherein the matrices are attached to CD3 agonists and CD28 agonists, wherein the nanomatrix provides activation signals to the population of TILs, thereby activating and inducing the population of TILs to proliferate, wherein each matrix is 1 to 500 nm in length in its largest dimension, and wherein the method does not comprise the use of feeder cells during expansion of the population of TILs.

3611. The method of claim 3510, wherein the population of TILs contacted with the nanomatrix further comprises tumor cells.

~~37. The method of any one of claims 35 or 36, wherein the population of TILs is isolated from a subject and contacted with the nanomatrix without an additional expansion process of the population of TILs prior to contacting the population of TILs with the nanomatrix.~~

3812. The method of ~~any one of claims 3510 to 3711~~, wherein the CD3 agonists and the CD28 agonists are attached to the same polymer chains or to different polymer chains.

~~39. The method of any one of claims 35 to 37, wherein the CD3 agonists and the CD28 agonists are attached to different polymer chains.~~

~~40. The method of any one of claims 35 to 39, wherein the CD3 agonists are attached to the nanomatrix at 25 µg per mg of nanomatrix.~~

~~41~~13. The method of any one of claims ~~278 to 30~~ and ~~35~~10 to ~~40~~12, wherein the nanomatrix further comprises magnetic, paramagnetic or superparamagnetic nanocrystals embedded among or within the matrices of polymer chains.

~~42~~14. The method of any one of claims ~~278 to 30~~ and ~~35~~10 to ~~41~~13, wherein the matrix of polymer chains comprises a polymer of dextran; and/or the polymer chains are colloidal polymer chains.

~~43. The method of any one of claims 27 to 30 and 35 to 42, wherein the polymer chains are colloidal polymer chains.~~

~~44~~15. The method of any one of claims ~~278 to 30~~ and ~~35~~10 to ~~43~~14, wherein:

the ratio of volume of nanomatrix to volume of TILs is greater than or equal to 1:5;
and/or

the ratio of number of matrices to TILs is greater than or equal to 1:500.

~~45. The method of any one of claims 27 to 30 and 35 to 44, wherein the ratio of number of matrices to TILs is greater than or equal to 1:500.~~

~~46. The method of any one of claims 27 to 30 and 35 to 44, wherein the CD28 agonist is attached to the nanomatrix at 25 µg per mg of nanomatrix.~~

~~47. The method of any one of the preceding claims, wherein the agonists are recombinant agonists.~~

~~48~~16. The method of any one of the preceding claims, wherein the agonists are antibodies, optionally humanized antibodies.

~~49. The method of claim 48, wherein the antibodies are humanized antibodies.~~

~~50~~17. The method of any one of claims ~~278 to 30~~ and ~~35~~10 to ~~49~~16, wherein the CD3 agonist is OKT3 or UCHT1.

~~51. The method of any of one of claims 35 to 50, wherein the TILs to be expanded are from a subject who had previously submitted a sample of TILs for expansion, wherein the previous TIL expansion comprises a pre-REP step and wherein the number of TILs isolated from the pre-REP step was less than 1000 TILs.~~

~~52. The method of any of one of claims 35 to 50, wherein the TILs to be expanded are from a subject who had previously submitted a sample of TILs for expansion, wherein the previous~~

~~TIL expansion comprises a pre-REP step and wherein the fold expansion of TILs isolated from the pre-REP step was less than 5 fold.~~

~~5318.~~ A method for expanding a population of TILs comprising contacting the population of TILs with a composition comprising a first, a second, and a third soluble monospecific complex, wherein each soluble monospecific complex comprises two antibodies or fragments thereof linked together, wherein each antibody or fragments thereof of each soluble monospecific complex specifically binds to the same antigen on the population of TILs, wherein the first soluble monospecific complex comprises an anti-CD3 antibody, wherein the second soluble monospecific complex comprises an anti-CD28 antibody, and wherein the third soluble monospecific complex comprises an anti-CD2 antibody, and the method does not comprise the use of feeder cells during expansion of the population of TILs.

~~5419.~~ The method of claim ~~5318~~, wherein the population of TILs contacted with the composition further comprises tumor cells.

~~55.—The method of any one of claims 53 or 54, wherein the population of TILs is isolated from a subject and contacted with the composition without an additional expansion process of the population of TILs prior to contacting the population of TILs with the composition.~~

~~5620.~~ The method of ~~any one of claims 5318 to 5519~~, wherein the soluble monospecific complexes are at a concentration of 0.2-25 µl/ml.

~~5721.~~ The method of any one of claims ~~5318~~ to ~~5620~~, wherein the soluble monospecific complexes are tetrameric antibody complexes (TACs optionally comprising two antibodies from a first animal species bound by two antibody molecules from a second species that specifically bind to the Fc portion of the antibodies from the first animal species.

~~58.—The method of claim 57, wherein each TAC comprises two antibodies from a first animal species bound by two antibody molecules from a second species that specifically bind to the Fc portion of the antibodies from the first animal species.~~

~~5922.~~ The method of any one of claims ~~5318~~ to ~~5821~~, wherein the anti-CD3 antibody is OKT3 or UCHT1.

~~60.—The method of any of one of claims 53 to 59, wherein the TILs to be expanded are from a subject who had previously submitted a sample of TILs for expansion, wherein the previous TIL expansion comprises a pre-REP step and wherein the number of TILs isolated from the pre-REP step was less than 1000 TILs.~~

~~61. The method of any of one of claims 53 to 59, wherein the TILs to be expanded are from a subject who had previously submitted a sample of TILs for expansion, wherein the previous TIL expansion comprises a pre-REP step and wherein the fold expansion of TILs isolated from the pre-REP step was less than 5 fold.~~

~~6223. The method of any one of claims 3510 to 4012 and 5318 to 5922, further comprising contacting the population of TILs with the cytokine IL-2, optionally at a time interval selected from the group consisting of 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days.~~

~~63 The method of claim 62, wherein the TILs are contacted with the cytokine IL-2 at a time interval selected from the group consisting of 1 day, 2 days, 3 days, 4 days, 5 days, and 6 days.~~

~~6424. The method of claim 6223 or 63, wherein the final concentration of the cytokine IL-2 is 100 U/ml to 7,000 U/ml.~~

~~6525. The method of any one of the preceding claims, wherein the TILs are expanded:~~

~~_____ from an initial population of TILs of 100 to 100,000 TILs; and/or~~

~~_____ for up to 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 days, or for 9-25 days, 9-21 days, or 9-14 days; and/or~~

~~_____ 500 to 500,000-fold.~~

~~66. The method of any one of the preceding claims, wherein the TILs are expanded for 9-25 days, 9-21 days, or 9-14 days.~~

~~67. The method of any one of the preceding claims, wherein the TILs are expanded 500 to 500,000 fold.~~

~~68. The method of any one of the preceding claims, wherein the population of TILs is expanded from an initial population of TILs of 100 to 100,000 TILs.~~

~~69. The method of any one of claims 65 to 68, wherein the population of TILs is expanded at least 1,500-fold at day 14 of the expansion.~~

~~70. The method of claim 69, wherein the population of TILs is expanded at most 100,000-fold at day 14 of expansion.~~

~~71. The method of any one of claims 65 to 68, wherein the population of TILs is expanded at least 15,000-fold at day 21 of the expansion.~~

~~72.—The method of claim 71, wherein the population of TILs is expanded at most 500,000-fold at day 21 of expansion.~~

~~73.—The method of any one of the preceding claims, wherein members of the population of TILs are genetically modified.~~

~~74.—The method of any one of the preceding claims, wherein members of the population of TILs are modified by a gene-regulating system.~~

~~75.—The method of claim 74, wherein the members of the population of TILs are modified using RNA interference.~~

~~76.—The method of claim 74, wherein the members of the population of TILs are modified using a transcription activator-like effector nuclease (TALEN).~~

~~77.—The method of claim 74, wherein the members of the population of TILs are modified using a zinc-finger nuclease.~~

~~78.—The method of claim 74, wherein the members of the population of TILs are modified using an RNA-guided nuclease.~~

~~79.—The method of claim 74, wherein members of the population of TILs are modified using a Cas enzyme and at least one guide RNA.~~

~~80.—The method of claim 79, wherein the Cas enzyme is Cas9.~~

~~81.—The method of any one of claims 74-80, wherein members of the population of TILs are modified at one or more genes selected from the group consisting of *ANKRD11*, *BCL2L1*, *BCL3*, *BCOR*, *CALM2*, *CBLB*, *CHIC2*, *CTLA4*, *DHODH*, *E2F8*, *EGR2*, *FLI1*, *FOXP3*, *GATA3*, *GNAS*, *HAVCR2*, *IKZF1*, *IKZF2*, *IKZF3*, *LAG3*, *MAP4K*, *NFKB1A*, *NR4A3*, *NRP1*, *PBRM1*, *PCBP1*, *PDCD1*, *PELI1*, *PIK3CD*, *PPP2R2D*, *PTPN1*, *PTPN2*, *PTPN22*, *PTPN6*, *RBM39*, *RC3H1*, *SEMA7A*, *SERPINA3*, *SETD5*, *SH2B3*, *SH2D1A*, *SMAD2*, *SOC31*, *TANK*, *TGFBRI*, *TGFBR2*, *TIGIT*, *TNFAIP3*, *TNIP1*, *TRAF6*, *UMPS*, *WDR6* and *ZC3H12A*.~~

~~82.—The method of claim 81, wherein the modification at one or more genes is an insertion, deletion, or mutation of one or more nucleic acids.~~

83. — ~~The method of any one of claims 81 or 82, wherein the modification at one or more genes results in the reduction or inhibition of expression of the gene and/or function of a protein encoded by the gene.~~

84. — ~~The method of claim 74, wherein members of the population of TILs are epigenetically modified.~~

85. — ~~The method of claim 84, wherein the epigenetic modification is a histone modification.~~

86. — ~~The method of claim 84, wherein members of the population of TILs are modified at one or more genes selected from the group consisting of *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*, *PELH*, *PIK3CD*, *PPP2R2D*, *PTPN1*, *PTPN2*, *PTPN22*, *PTPN6*, *RBM39*, *RC3H1*, *SEMA7A*, *SERPINA3*, *SETD5*, *SH2B3*, *SH2D1A*, *SMAD2*, *SOCS1*, *TANK*, *TGFBRI*, *TGFBRI2*, *TIGIT*, *TNFAIP3*, *TNIP1*, *TRAF6*, *UMPS*, *WDR6* and *ZC3H12A*.~~

87. — ~~The method of claim 86, wherein the modification at one or more genes is methylation of one or more nucleic acids.~~

88. — ~~The method of any one of claims 86 or 87, wherein the modification at one or more genes results in the reduction or inhibition of expression of the gene and/or function of a protein encoded by the gene.~~

89. — ~~The method of any one of claims 86 to 88, wherein members of the population of TILs are modified at the *SOCS1* gene.~~

90. — ~~The method of claim 89, wherein the modification of the *SOCS1* gene results in the reduction or inhibition of expression of the gene and/or function of a protein encoded by the gene.~~

91. — ~~The method of any one of claims 74 to 90, wherein members of the population of TILs are modified at more than one gene.~~

92. — ~~The method of claim 91, wherein members of the population of TILs are modified at the *SOCS1* gene and one or more additional genes selected from the group consisting of *PTPN2*, *ZC3H12A*, *CBLB*, *RC3H1*, *NFKBIA*.~~

93. — ~~The method of claim 91, wherein members of the population of TILs are modified at the *SOCS1* and *ZC3H12A* genes.~~

94. — ~~The method of claim 93, wherein the modification of the *SOCS1* and *ZC3H12A* genes results in the reduction or inhibition of expression of the genes and/or function of proteins encoded by the gene.~~

95. — ~~The method of claim 91, wherein members of the population of TILs are modified at the *SOCS1* and *CBLB* genes.~~

96. — ~~The method of claim 93, wherein the modification of the *SOCS1* and *CBLB* genes results in the reduction or inhibition of expression of the genes and/or function of proteins encoded by the gene.~~

97. — ~~The method of claim 91, wherein members of the population of TILs are modified at the *SOCS1* and *RC3H1* genes.~~

98. — ~~The method of claim 93, wherein the modification of the *SOCS1* and *RC3H1* genes results in the reduction or inhibition of expression of the genes and/or function of proteins encoded by the gene.~~

99. — ~~The method of claim 91, wherein members of the population of TILs are modified at the *SOCS1* and *NFKB1A* genes.~~

100. — ~~The method of claim 93, wherein the modification of the *SOCS1* and *NFKB1A* genes results in the reduction or inhibition of expression of the genes and/or function of proteins encoded by the gene.~~

101. — ~~The method of any one of the preceding claims, wherein the population of TILs is expanded to produce an expanded population of TILs, wherein at least 10% of the expanded population have a central memory T cell phenotype.~~

102. — ~~The method of any one of the preceding claims, wherein the population of TILs is expanded to produce an expanded population of TILs, wherein at least 15% of the expanded population have a central memory T cell phenotype.~~

~~103. The method of any one of claims 1 to 100, wherein the population of TILs is expanded to produce an expanded population of TILs, wherein 5 to 50% of the expanded population have a central memory T cell phenotype at day 14 of expansion.~~

~~104. The method of any one of claims 1 to 100, wherein the population of TILs is expanded to produce an expanded population of TILs, wherein 10 to 25% of the expanded population have a central memory T cell phenotype at day 14 of expansion.~~

~~105~~26. A composition comprising an expanded population of TILs produced by the method of any one of the preceding claims.