CLAIMS

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 any one of the preceding claims,

wherein the disaggregated tumor sample comprises tumor fragments that are 0.5 to 4 mm^3 in size; and/or

wherein the disaggregated tumor sample comprises digested tumor fragments.

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

5. The method of claim 4, 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.

6. The method of claim 5, wherein:

the TCR agonist is a CD3 agonist; and/or

the CD3 agonist is OKT3; and/or

the CD28 agonist is CD86.

7. The method of any one of claims 1 to 3, wherein the medium does not comprise feeder cells.

8. 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.

9. The method of any one of claims 1 to 7, 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.

10. 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.

11. The method of claim 10, wherein the population of TILs contacted with the nanomatrix further comprises tumor cells.

12. The method of claim 10 or 11, wherein the CD3 agonists and the CD28 agonists are attached to the same polymer chains or to different polymer chains.

13. The method of any one of claims 8 and 10 to 12, wherein the nanomatrix further comprises magnetic, paramagnetic or superparamagnetic nanocrystals embedded among or within the matrices of polymer chains.

14. The method of any one of claims 8 and 10 to 13, wherein the matrix of polymer chains comprises a polymer of dextran; and/or the polymer chains are colloidal polymer chains.

15. The method of any one of claims 8 and 10 to 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.

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

17. The method of any one of claims 8 and 10 to 16, wherein the CD3 agonist is OKT3 or UCHT1.

18. 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.

19. The method of claim 18, wherein the population of TILs contacted with the composition further comprises tumor cells.

20. The method of claim 18 or 19, wherein the soluble monospecific complexes are at a concentration of 0.2-25 μl/ml.

21. The method of any one of claims 18 to 20, 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.

22. The method of any one of claims 18 to 21, wherein the anti-CD3 antibody is OKT3 or UCHT1.

23. The method of any one of claims 10 to 12 and 18 to 22, 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.

24. The method of claim 23, wherein the final concentration of the cytokine IL-2 is 100 U/ml to 7,000 U/ml.

25. 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.

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