

CLAIMS

WHAT IS CLAIMED IS:

1. An *ex vivo* method for selectively activating and expanding $\gamma\delta$ T cells in an isolated mixed cell population, the method comprising contacting the isolated mixed cell population with one or more soluble multivalent agents that selectively activate and expand $\gamma\delta$ T cells by binding to at least one epitope of a $\gamma\delta$ TCR, wherein the soluble multivalent agent is at least tetravalent.
2. An *ex vivo* method for selectively activating and expanding one or more $\gamma\delta$ T cell subtypes in an isolated mixed cell population, the method comprising contacting the isolated mixed cell population with one or more soluble multivalent agents that selectively activate and expand $\delta 1$ T cells, $\delta 2$ T cells, or $\delta 3$ T cells, or a combination thereof, preferably wherein the one or more agents that selectively activate and expand $\delta 1$ T cells bind to an activating epitope specific of a $\delta 1$ TCR; the one or more agents that selectively activate and expand $\delta 2$ T cells bind to an activating epitope specific of a $\delta 2$ TCR; and the one or more agents that selectively activate and expand $\delta 3$ T cells bind to an activating epitope specific of a $\delta 3$ TCR, thereby activating and expanding the desired $\gamma\delta$ T cell subtype in the mixed cell population, wherein the soluble multivalent agent is at least tetravalent.
3. An *in vitro* and/or *ex vivo* method for producing an enriched $\gamma\delta$ T-cell population comprising directly contacting an isolated mixed cell population comprising $\gamma\delta$ T-cells, or a purified fraction thereof, with one or more soluble multivalent agents; preferably wherein the soluble multivalent agent(s) activate and expand $\gamma\delta$ T cells by binding to at least one epitope of a $\gamma\delta$ TCR, wherein the soluble multivalent agent is at least tetravalent.
4. An *in vitro* and/or *ex vivo* method for producing enriched $\gamma\delta$ T-cell sub-populations from isolated mixed cell populations, comprising directly contacting an isolated mixed cell population with one or more soluble multivalent agents that i) selectively expand $\delta 1$ T-cells by binding to an epitope specific of a $\delta 1$ TCR, ii) that selectively expand $\delta 2$ T cells by binding to an epitope specific of a $\delta 2$ TCR, and iii) that selectively expand $\delta 3$ T cells by binding to an epitope specific of a $\delta 3$ TCR, to provide an enriched $\gamma\delta$ T cell sub-population, wherein the soluble multivalent agent is at least tetravalent.

5. The method according to any preceding claim, wherein the soluble multivalent agent comprises at least two antigen-binding sites that specifically bind the same antigen, or wherein the multivalent agent comprises at least two antigen-binding sites that specifically bind the same epitope of the same antigen.

~~6. The method according to claim 5, wherein the soluble multivalent agent is, or is at least, bivalent, trivalent, or tetravalent, and optionally monospecific.~~

~~7. The method according to any preceding claim, wherein the soluble multivalent agent is tetravalent.~~

~~8.6.~~ The method according to any preceding claim, wherein the soluble multivalent agent is monospecific.

~~9.7.~~ The method according to claim 5, wherein the antigen-binding sites bind to different epitopes on the constant or variable regions of δ TCR and/or γ TCR.

~~10.8.~~ The method according to claim 5, wherein the antigen-binding sites comprise the CDRs from $\gamma\delta$ TCR pan MAbs, preferably wherein the antigen-binding sites specifically bind domains shared by different γ and δ TCRs on either the γ or δ chain or both, including $\delta 1$, $\delta 2$, and $\delta 3$ T cell populations.

~~11.9.~~ The method according to claim 5, wherein the soluble multivalent agent selectively expands $\delta 1$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 1$ variable region.

~~12.10.~~ The method according to claim ~~11.9~~, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 1$ TCR Bin 1 $\delta 1$ epitope, Bin 1b $\delta 1$ epitope, Bin 2 $\delta 1$ epitope, Bin 2b $\delta 1$ epitope, Bin 2c $\delta 1$ epitope, Bin 3 $\delta 1$ epitope, Bin 4 $\delta 1$ epitope, Bin 5 $\delta 1$ epitope, Bin 6 $\delta 1$ epitope, Bin 7 $\delta 1$ epitope, Bin 8 $\delta 1$ epitope, or a Bin 9 $\delta 1$ epitope of a human $\delta 1$ TCR.

~~13.11.~~ The method according to claim ~~11.9~~, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 1$ -05, $\delta 1$ -08,

δ1-18, δ1-22, δ1-23, δ1-26, δ1-35, δ1-37, δ1-39, δ1-113, δ1-143, δ1-149, δ1-155, δ1-182, δ1-183, δ1-191, δ1-192, δ1-195, δ1-197, δ1-199, δ1-201, δ1-203, δ1-239, δ1-253, δ1-257, δ1-278, δ1-282, and δ1-285.

~~14.12.~~ The method according to claim ~~11~~9, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ1-05, δ1-08, δ1-18, δ1-22, δ1-23, δ1-26, δ1-35, δ1-37, δ1-39, δ1-113, δ1-143, δ1-149, δ1-155, δ1-182, δ1-183, δ1-191, δ1-192, δ1-195, δ1-197, δ1-199, δ1-201, δ1-203, δ1-239, δ1-253, δ1-257, δ1-278, δ1-282, and δ1-285.

~~15.13.~~ The method of claim ~~11~~9, wherein the soluble multivalent agent comprises the CDRs of antibody δ1-35 or δ1-08, or binds the same epitope as antibody δ1-08 or δ1-35.

~~16.14.~~ The method according to claim ~~11~~9, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind the same epitope as an antibody selected from TS-1 and TS8.2.

~~17.15.~~ The method according to claim ~~16~~14, wherein the soluble multivalent agent comprises the CDRs of TS-1 or TS8.2 and/or is a humanized TS-1 or TS8.2.

~~18.16.~~ The method according to claim ~~11~~9, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising residues Arg71, Asp72 and Lys120 of the δ1 variable region.

~~19.17.~~ The method according to claim 5, wherein the soluble multivalent agent selectively expands δ1 T-cells and δ3 T cells.

~~20.18.~~ The method according to claim 5, wherein the soluble multivalent agent selectively expands δ1 T cells selectively expands δ1, δ3, δ4, and δ5 γδ T cells.

~~21.19.~~ The method according to claim 5, wherein the soluble multivalent agent selectively expands δ2 T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a δ2 variable region.

~~22~~20. The method according to claim ~~24~~19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant $\delta 1$ TCR polypeptide comprising a mutation at K120 of delta J1 and delta J2.

~~23~~21. The method according to claim ~~24~~19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 2$ TCR Bin 1 $\delta 2$ epitope, Bin 2 $\delta 2$ epitope, Bin 3 $\delta 2$ epitope, or Bin 4 $\delta 2$ epitope of a human $\delta 2$ TCR.

~~24~~22. The method according to claim ~~24~~19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.

~~25~~23. The method according to claim ~~24~~22, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.

~~26~~24. The method of claim ~~24~~19, wherein the soluble multivalent agent comprises the CDRs of antibody $\delta 2$ -37 or binds the same epitope as antibody $\delta 2$ -37.

~~27~~25. The method according to claim ~~24~~19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same epitope as an antibody selected from 15D and B6.

~~28~~26. The method according to claim ~~26~~24, wherein the soluble multivalent agent comprises the CDRs of antibody 15D or B6 and/or is a humanized 15D and B6 antibody.

~~29~~27. The method according to claim ~~24~~19, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant $\delta 2$ TCR polypeptide comprising a mutation at G35 of the $\delta 2$ variable region.

~~30~~28. The method according to claim 5, wherein the soluble multivalent agent selectively expands $\delta 3$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 3$ variable region.

~~31.29.~~ The method according to claim ~~3028~~, wherein the soluble multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47 and $\delta 3$ -58.

~~32.30.~~ The method according to claim ~~3129~~, wherein the soluble multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47 and $\delta 3$ -58.

~~33.31.~~ The method according to any preceding claim, wherein the method comprises simultaneously or sequentially culturing the $\gamma\delta$ T-cell population with a cytokine, preferably wherein the cytokine is a common gamma chain cytokine.

~~34.32.~~ The method according to claim ~~3331~~, wherein the cytokine is selected from the group consisting of IL-2, IL-7, IL-9, IL-12, IL-15, IL-18, IL-19, IL-21, IL 23, and IL-33, preferably wherein the cytokine is IL-2, IL-15, IL-12, or IL-21.

~~35.33.~~ The method according to any preceding claim, wherein the $\gamma\delta$ T-cell population is engineered before and/or after activation and/or expansion.

~~36.34.~~ The method according to claim ~~3533~~, wherein the $\gamma\delta$ T-cell population is engineered to stably express one or more tumor recognition moieties encoded by expression cassettes, and/or wherein the $\gamma\delta$ T-cell population is engineered to stably express a transgene that encodes at least one secreted cytokine.

~~37.35.~~ The method according to claim ~~3634~~, wherein the at least one secreted cytokine is a common gamma chain cytokine selected from the group consisting of IL-2, IL-15 and IL-4, preferably wherein the cytokine is IL-2, IL-15 or IL-4.

~~38.~~ ~~The method according to any preceding claim, wherein the method further comprises administering the expanded / enriched engineered and/or non-engineered $\gamma\delta$ T cell populations to a patient in need thereof, preferably wherein the administered population of $\gamma\delta$ T cells comprises at least 60% $\gamma\delta$ T cells.~~

~~39. The method according to claim 37, wherein the administered population of engineered and/or non-engineered $\gamma\delta$ T cells is a population of cells that are autologous to the subject.~~

~~40. The method according to claim 37, wherein the administered population of engineered and/or non-engineered $\gamma\delta$ T cells is a population of cells that are allogeneic to the subject.~~

~~41. The method according to any preceding claim, further comprising performing a depletion step for $\alpha\beta$ T cells after activation and expansion of the $\gamma\delta$ T-cell population, and before administration to the subject.~~

~~42.36. A soluble composition comprising one or more multivalent agent(s) for selectively activating and expanding $\gamma\delta$ T cells, a subtype thereof, or combinations thereof, wherein said multivalent agent(s) comprises at least two antigen-binding sites that specifically bind the same epitope of a $\gamma\delta$ TCR; preferably wherein the multivalent agent is, ~~or is~~ at least, bivalent, trivalent, tetravalent, ~~or pentavalent, and optionally monospecific.~~~~

~~43. The soluble composition according to claim 42, wherein the multivalent agent is tetravalent.~~

~~44.37. The soluble composition according to claim 42 ~~or 43~~36, wherein the multivalent agent is monospecific.~~

~~45.38. The soluble composition according to claim ~~42~~36, wherein the antigen-binding sites comprise the CDRs from $\gamma\delta$ TCR pan MAbs, preferably wherein the multivalent agent comprises the amino acid sequence set forth in Fig. x or y.~~

~~46.39. The soluble composition according to claim ~~42~~36, wherein the multivalent agent selectively expands $\delta 1$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 1$ variable region.~~

~~47.40. The soluble composition according to claim ~~46~~39, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 1$ TCR Bin 1 $\delta 1$ epitope, Bin 1b $\delta 1$ epitope, Bin 2 $\delta 1$ epitope, Bin 2b $\delta 1$ epitope, Bin 2c $\delta 1$ epitope, Bin 3 $\delta 1$ epitope,~~

Bin 4 δ 1 epitope, Bin 5 δ 1 epitope, Bin 6 δ 1 epitope, Bin 7 δ 1 epitope, Bin 8 δ 1 epitope, or a Bin 9 δ 1 epitope of a human δ 1 TCR.

~~48.41.~~ The soluble composition according to claim ~~4639~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of δ 1-05, δ 1-08, δ 1-18, δ 1-22, δ 1-23, δ 1-26, δ 1-35, δ 1-37, δ 1-39, δ 1-113, δ 1-143, δ 1-149, δ 1-155, δ 1-182, δ 1-183, δ 1-191, δ 1-192, δ 1-195, δ 1-197, δ 1-199, δ 1-201, δ 1-203, δ 1-239, δ 1-253, δ 1-257, δ 1-278, δ 1-282, and δ 1-285.

~~49.42.~~ The soluble composition according to claim ~~4639~~, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of δ 1-05, δ 1-08, δ 1-18, δ 1-22, δ 1-23, δ 1-26, δ 1-35, δ 1-37, δ 1-39, δ 1-113, δ 1-143, δ 1-149, δ 1-155, δ 1-182, δ 1-183, δ 1-191, δ 1-192, δ 1-195, δ 1-197, δ 1-199, δ 1-201, δ 1-203, δ 1-239, δ 1-253, δ 1-257, δ 1-278, δ 1-282, and δ 1-285.

~~50.43.~~ The soluble composition of claim ~~4639~~, wherein the multivalent agent comprises the CDRs of antibody δ 1-35 or δ 1-08, or binds the same epitope as antibody δ 1-08 or δ 1-35, preferably wherein the multivalent agent comprises the amino acid sequence set forth in Fig. XX.

~~51.44.~~ The soluble composition according to claim ~~4639~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind the same epitope as an antibody selected from TS-1 and TS8.2.

~~52.45.~~ The soluble composition according to claim ~~5144~~, wherein the soluble multivalent agent comprises the CDRs of TS-1 or TS8.2 and/or is a humanized TS-1 or TS8.2.

~~53.46.~~ The soluble composition according to claim ~~4639~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising residues Arg71, Asp72 and Lys120 of the δ 1 variable region.

~~54.47.~~ The soluble composition according to claim ~~4236~~, wherein the multivalent agent selectively expands δ 1 T-cells and δ 3 T cells.

~~55.48.~~ The soluble composition according to claim ~~4236~~, wherein the multivalent agent selectively expands $\delta 1$ T cells selectively expands $\delta 1$, $\delta 3$, $\delta 4$, and $\delta 5$ $\gamma\delta$ T cells.

~~56.49.~~ The soluble composition according to claim ~~4236~~, wherein the multivalent agent selectively expands $\delta 2$ T-cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 2$ variable region.

~~57.50.~~ The soluble composition according to claim ~~5649~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant $\delta 1$ TCR polypeptide comprising a mutation at K120 of delta J1 and delta J2.

~~58.51.~~ The soluble composition according to claim ~~5649~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind a $\delta 2$ TCR Bin 1 $\delta 2$ epitope, Bin 2 $\delta 2$ epitope, Bin 3 $\delta 2$ epitope, or Bin 4 $\delta 2$ epitope of a human $\delta 2$ TCR.

~~59.52.~~ The soluble composition according to claim ~~5649~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.

~~60.53.~~ The soluble composition according to claim ~~5952~~, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 2$ -14, $\delta 2$ -17, $\delta 2$ -22, $\delta 2$ -30, $\delta 2$ -31, $\delta 2$ -32, $\delta 2$ -33, $\delta 2$ -35, $\delta 2$ -36, and $\delta 2$ -37.

~~61.54.~~ The soluble composition of claim ~~5649~~, wherein the multivalent agent comprises the CDRs of antibody $\delta 2$ -37 or binds the same epitope as antibody $\delta 2$ -37.

~~62.55.~~ The soluble composition according to claim ~~5649~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same epitope as an antibody selected from 15D and B6.

~~63.56.~~ The soluble composition according to claim ~~6255~~, wherein the multivalent agent comprises the CDRs of antibody 15D or B6 and/or is a humanized 15D and B6 antibody.

~~64.57.~~ The soluble composition according to claim ~~5649~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that have reduced binding to a mutant $\delta 2$ TCR polypeptide comprising a mutation at G35 of the $\delta 2$ variable region.

~~65.58.~~ The soluble composition according to claim ~~4236~~, wherein the multivalent agent selectively expands $\delta 3$ T cells and comprises at least two, or greater than two, antigen-binding sites that specifically bind to an epitope comprising a $\delta 3$ variable region.

~~66.59.~~ The soluble composition according to claim ~~6558~~, wherein the multivalent agent comprises at least two, or greater than two, antigen-binding sites that specifically bind to the same, or essentially the same, epitope as, or competes with, an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47 and $\delta 3$ -58.

~~67.60.~~ The soluble composition according to claim ~~6659~~, wherein the multivalent agent comprises the CDRs of an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47 and $\delta 3$ -58.

~~68.61.~~ Use of a soluble composition according to any one of claims ~~4236-6760~~ in the manufacture of a medicament for treating a cancer, infectious disease, inflammatory disease, or an autoimmune disease in a subject in need thereof, wherein the medicament comprises the resulting expanded $\gamma\delta$ T cell population, subtype thereof, or admixtures thereof.