

CLAIMS

What is claimed is:

1. An antibody or fragment thereof that binds an epitope specific to a $\delta\gamma$ TCR₂,
wherein the antibody or fragment thereof comprises:
 - (i) a heavy chain variable region/light chain variable region (HCVR/LCVR) sequence pair selected from the group consisting of SEQ ID NOs: 1/8, 2/9, 3/10, 4/11, 5/12, 6/13, and 7/14; or
 - (ii) the six complementarity determining regions (CDRs) of a HCVR/LCVR sequence pair selected from the group consisting of SEQ ID NOs: 1/8, 2/9, 3/10, 4/11, 5/12, 6/13, and 7/14.
2. The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof ~~binds essentially the same epitope as~~ comprises:
 - a) the six CDRs of a HCVR/LCVR sequence pair selected from the group consisting of: 1/8, 3/10, 4/11, 5/12, 6/13, and 7/14;
 - b) the six CDRs of a HCVR/LCVR sequence pair selected from the group consisting of: 1/8, 3/10, 5/12, and 7/14;
 - c) the six CDRs of a HCVR/LCVR sequence pair selected from the group consisting of: 3/10, 5/12, and 7/14;
 - d) the six CDRs of a HCVR/LCVR sequence pair selected from the group consisting of: 4/11 and 5/12;
 - e) the six CDRs of a HCVR/LCVR sequence pair consisting of SEQ ID NOs: 1/8; or
 - f) the six CDRs of a HCVR/LCVR sequence pair consisting of SEQ ID NOs: 4/11.
 - a) ~~an antibody selected from the group consisting of $\delta\gamma$ -08, $\delta\gamma$ -20, $\delta\gamma$ -23, $\delta\gamma$ -31, $\delta\gamma$ -42, $\delta\gamma$ -47, and $\delta\gamma$ -58;~~
 - b) ~~an antibody selected from the group consisting of $\delta\gamma$ -08, $\delta\gamma$ -23, $\delta\gamma$ -31, $\delta\gamma$ -42, $\delta\gamma$ -47, and $\delta\gamma$ -58;~~
 - c) ~~an antibody selected from the group consisting of $\delta\gamma$ -08, $\delta\gamma$ -23, $\delta\gamma$ -42, and $\delta\gamma$ -58;~~
 - d) ~~an antibody selected from the group consisting of $\delta\gamma$ -23, $\delta\gamma$ -42, and $\delta\gamma$ -58;~~

e) ~~an antibody selected from the group consisting of $\delta 3-31$ and $\delta 3-42$;~~

f) ~~antibody $\delta 3-08$; or~~

g) ~~antibody $\delta 3-31$.~~

3. ~~The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof binds the same epitope as:~~

a) ~~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 $\delta 3-58$;~~

b) ~~an antibody selected from the group consisting of $\delta 3-08$, $\delta 3-23$, $\delta 3-31$, $\delta 3-42$, $\delta 3-47$, and $\delta 3-58$;~~

c) ~~an antibody selected from the group consisting of $\delta 3-08$, $\delta 3-23$, $\delta 3-42$, and $\delta 3-58$;~~

d) ~~an antibody selected from the group consisting of $\delta 3-23$, $\delta 3-42$, and $\delta 3-58$;~~

e) ~~an antibody selected from the group consisting of $\delta 3-31$ and $\delta 3-42$;~~

f) ~~antibody $\delta 3-08$; or~~

g) ~~antibody $\delta 3-31$.~~

4. ~~The antibody or fragment thereof of claim 1, wherein the antibody or fragment thereof comprises the complementarity determining regions of:~~

a) ~~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 $\delta 3-58$;~~

b) ~~an antibody selected from the group consisting of $\delta 3-08$, $\delta 3-23$, $\delta 3-31$, $\delta 3-42$, $\delta 3-47$, and $\delta 3-58$;~~

c) ~~an antibody selected from the group consisting of $\delta 3-08$, $\delta 3-23$, $\delta 3-42$, and $\delta 3-58$;~~

d) ~~an antibody selected from the group consisting of $\delta 3-23$, $\delta 3-42$, and $\delta 3-58$;~~

e) ~~an antibody selected from the group consisting of $\delta 3$ -31 and $\delta 3$ -42;~~

f) ~~antibody $\delta 3$ -08; or~~

g) ~~antibody $\delta 3$ -31.~~

35. The antibody or fragment thereof of claim 1 or 2, wherein the antibody or fragment thereof specifically binds to V $\delta 3$ of a $\gamma\delta$ TCR;

~~6. The antibody of claim 5, optionally~~ wherein the antibody binds to:

i) a region distal from the γ -chain binding interface of V $\delta 3$;

~~7. The antibody of claim 5, wherein the antibody binds to ii)~~ β strand D and E of the V $\delta 3$ of the $\gamma\delta$ TCR according to IMGT nomenclature;

~~8. The antibody of claim 5, wherein the antibody binds to iii)~~ β strand C'' and D and a loop between β strands E and F of the V $\delta 3$ of the $\gamma\delta$ TCR according to IMGT nomenclature; or

~~9. The antibody of claim 5, wherein the antibody binds to iv)~~ β strand A, B, D, and E of the V $\delta 3$ of the $\gamma\delta$ TCR according to IMGT nomenclature.

~~10. The antibody or fragment thereof of any one of claims 1-9, wherein the antibody or fragment thereof selectively expands a $\delta 3$ - $\gamma\delta$ T cell as compared to $\alpha\beta$ T cells in a mixed cell population comprising $\gamma\delta$ T cells and $\alpha\beta$ T cells.~~

411. The antibody or fragment thereof of any one of claims 1-~~310~~, wherein the antibody or fragment thereof is bound to a $\delta 3$ $\gamma\delta$ TCR;

~~12. The antibody or fragment thereof of claim 11; optionally~~ wherein the $\delta 3$ $\gamma\delta$ TCR is expressed on the surface of a $\delta 3$ $\gamma\delta$ T cell.

513. The antibody or fragment thereof any one of claims 1-~~123~~, wherein the antibody or fragment thereof is bound to an extracellular surface of an antigen presenting cell (APC), such as an artificial antigen presenting cell (aAPC).

614. The antibody or fragment thereof of claim ~~135~~, wherein the antibody or fragment thereof is anchored in the membrane of the APC or aAPC;

~~15. The antibody, or fragment thereof of claim 13,~~ wherein an Fc region of the antibody or fragment thereof is bound to an Fc-receptor expressed by the APC or aAPC.

716. The antibody or fragment thereof of any one of claims 1-~~156~~, wherein the antibody or fragment thereof selectively expands a $\delta\gamma$ T cell as compared to $\alpha\beta$ T cells in a mixed cell population comprising $\gamma\delta$ T cells and $\alpha\beta$ T cells. ~~competes for binding to:~~

- ~~a) an antibody selected from the group consisting of $\delta\delta$ -08, $\delta\delta$ -20, $\delta\delta$ -23, $\delta\delta$ -31, $\delta\delta$ -42, $\delta\delta$ -47, and $\delta\delta$ -58;~~
- ~~b) an antibody selected from the group consisting of $\delta\delta$ -08, $\delta\delta$ -23, $\delta\delta$ -31, $\delta\delta$ -42, $\delta\delta$ -47, and $\delta\delta$ -58;~~
- ~~c) an antibody selected from the group consisting of $\delta\delta$ -08, $\delta\delta$ -23, $\delta\delta$ -42, and $\delta\delta$ -58;~~
- ~~d) an antibody selected from the group consisting of $\delta\delta$ -23, $\delta\delta$ -42, and $\delta\delta$ -58;~~
- ~~e) an antibody selected from the group consisting of $\delta\delta$ -31 and $\delta\delta$ -42;~~
- ~~f) antibody $\delta\delta$ -08; or~~
- ~~g) antibody $\delta\delta$ -31.~~

~~17. The antibody or fragment thereof of any one of claims 1-16, wherein the antibody or fragment thereof is a specific activator of $\delta\gamma$ T cells.~~

18. A nucleic acid encoding any one of the antibodies or fragments thereof according to any one of claims 1-~~177~~, wherein the nucleic acid is operably linked to a heterologous promoter.

19. A host cell comprising any one of the antibodies or fragments thereof according to any one of claims 1-~~177~~ or a nucleic acid according to claim ~~188~~.

210. The host cell of claim ~~199~~, wherein the host cell is an artificial antigen presenting cell (aAPC).

211. A method of making an antibody or fragment thereof that binds an epitope specific to a $\delta\gamma$ TCR, the method comprising culturing the host cell of claim ~~1910~~ under conditions sufficient to produce the antibody or fragment thereof.

212. An *ex vivo* method for producing an enriched $\gamma\delta$ T cell population, comprising contacting a first cell population comprising $\gamma\delta$ T cells with one or more antibodies or fragments thereof according to claims 1-~~177~~.

213. The method of claim ~~2212~~, wherein the first cell population is an isolated mixed cell population comprising $\alpha\beta$ T cells and $\gamma\delta$ T cells;

~~24. The method of claim 23, optionally~~ wherein the first cell population comprises, or is, a sample of peripheral blood mononuclear cells (PBMCs);

~~25. The method of claim 23, or~~ wherein the first cell population is selected from a peripheral blood sample, a leukapheresis sample, a cord blood sample, a tumor sample, or a tissue sample.

1426. The method of ~~any one of claims 23 to 25~~ 12 or 13, wherein

i) the first cell population comprises less than 1% $\delta\delta$ $\gamma\delta$ T cells;

~~27. The method of any one of claims 22-26, wherein ii)~~ the first cell population comprises uncultured primary cells or unpassaged primary cells; and/or

~~28. The method of any one of claims 23-27, wherein iii)~~ the method comprises directly contacting the isolated mixed cell population with the one or more antibodies or fragments thereof.

15. The method of any one of claims 12 to 14, further comprising genetically engineering the $\gamma\delta$ T cells.

16. The method of claim 15, wherein the method comprises genetically engineering the enriched $\gamma\delta$ T cell population.

17. The method according to claim 15 or 16, wherein the $\gamma\delta$ T cells are engineered to express one or more tumor recognition moieties.

18. The method according to any one of claims 15-17, wherein:

a. the engineered $\gamma\delta$ T cells lack an HLA locus;

b. the engineered $\gamma\delta$ T cells are universal donor cells;

c. the engineered $\gamma\delta$ T cells are tumor-specific allogeneic $\gamma\delta$ T cells; or

d. the $\gamma\delta$ T cells are engineered to express two or more tumor recognition moieties.

19. The method according to claim 18(d):

i. wherein the two or more tumor recognition moieties are different, wherein each different tumor recognition moiety is engineered to recognize different epitopes of the same antigen; or

ii. wherein the two or more tumor recognition moieties are different, wherein each different tumor recognition moiety is engineered to recognize different epitopes of different antigens.

20. The method according to any one of claims 17-19, wherein:

a. the tumor recognition moiety is derived from a tumor infiltrating lymphocyte;

b. the tumor recognition moiety is cloned from a T-cell;

c. the tumor recognition moiety is an engineered T-cell receptor;

d. the tumor recognition moiety is an antibody, an antibody fragment, a scFv, a single domain antibody, a Fab, that recognizes a tumor antigen; or

e. the tumor antigen is a peptide-MHC complex, and the tumor recognition moiety recognizes the peptide-MHC complex.

21. The method according to claim 20(c), wherein:

i. the engineered T-cell receptor is derived from a human or a mouse T-cell receptor;

ii. the engineered T-cell receptor is an engineered $\alpha\beta$ TCR; or

iii. the engineered T-cell receptor is an engineered $\gamma\delta$ TCR.

22. The method according to any one of claims 12-16, wherein the $\gamma\delta$ T cells are engineered to express an antigen recognition moiety, wherein the antigen recognition moiety recognizes an antigen associated with an autoimmune disease, or wherein the antigen recognition moiety recognizes a pathogenic antigen, wherein the pathogenic antigen is derived from a pathogenic bacterium or a virus.

23. The method of any one of claims 12-22, wherein the first cell population is a first population of expanded $\gamma\delta$ T cells, optionally wherein the method comprises a first $\gamma\delta$ T cell expansion and a second $\gamma\delta$ T cell expansion.

24. The method of claim 13, further comprising a $\gamma\delta$ T cell expansion that yields the first cell population, wherein the first cell population is a population of expanded $\gamma\delta$ T cells.

25. The method of any one of claims 13-24, further comprising depleting $\alpha\beta$ T cells, B cells and/or NK cells in the enriched $\gamma\delta$ T-cell population.

~~29.~~ The method of claim 22, wherein the first cell population is a population comprising one or more engineered $\gamma\delta$ T cells.

~~30.~~ The method of any one of claims 22 or 29, wherein the first cell population is a first population of expanded $\gamma\delta$ T cells.

~~31.~~ The method of claim 30, wherein the method comprises a first $\gamma\delta$ T cell expansion and a second $\gamma\delta$ T cell expansion.

2632. The method of any one of claims 12-2522 to 31, wherein

i) the method comprises producing an enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta\gamma$ $\gamma\delta$ T cells.

~~33.~~ The method of claim ~~32~~, optionally wherein the enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta\gamma$ $\gamma\delta$ T cells is produced within 12 to 21 days; and/or

~~34.~~ The method of any one of claims ~~22 to 33~~, wherein ii) the method comprises expanding the $\delta\gamma$ $\gamma\delta$ T cells in the first cell population at least 1,000-fold, preferably at least 5,000-fold, ~~more preferably~~ or at least, or about, or at least about, 10,000 fold.

~~35.~~ The method of claim ~~34~~, optionally wherein the specified -fold expansion is achieved within 12 to 21 days.

2736. An ex vivo method for producing an enriched $\gamma\delta$ T cell population, comprising:

- a) contacting a first cell population comprising $\gamma\delta$ T cells with one or more first activating agents that activate and expand $\gamma\delta$ T cells, thereby producing an expanded first $\gamma\delta$ T cell population; and

b) contacting the expanded first $\gamma\delta$ T cell population with one or more second activating agents that activate and expand $\gamma\delta$ T cells, thereby producing the enriched $\gamma\delta$ T cell population,
wherein, at least one of the one or more first activating agents or at least one of the one or more second activating agents is an antibody or fragment thereof according to any one of claims 1-~~17~~7.

28~~37~~. The method of claim ~~36~~27, wherein

i) the method comprises isolating the expanded first $\gamma\delta$ T cell population after a) and before b); ~~); or~~

~~38. — The method of claim 36 or 37, wherein ii) a) comprises culturing the first cell population in the presence of an antigen presenting cell (APC) and the one or more first activating agents;~~

~~39. — The method of claim 36 or 37, wherein, or b) comprises culturing the expanded first $\gamma\delta$ T cell population in the presence of an antigen presenting cell (APC) and the one or more second activating agents.~~

29~~40~~. The method of ~~any one of claims 36-39~~claim 27 or 28, wherein the first cell population is:

i) an isolated mixed cell population comprising $\alpha\beta$ T cells and $\gamma\delta$ T cells;

~~41. — The method of claim 40, optionally wherein the first cell population is selected from a peripheral blood sample, a leukapheresis sample, a cord blood sample, a tumor sample, or a tissue sample;~~

~~42. — The method of claim 40, or wherein the first cell population comprises, or is, a sample of peripheral blood mononuclear cells (PBMCs); optionally wherein the first cell population comprises uncultured primary cells, unpassaged primary cells; or~~

ii) a population of engineered $\gamma\delta$ T cells.

~~43. — The method of any one of claims 36-42, wherein the first cell population comprises uncultured primary cells, unpassaged primary cells.~~

~~44. The method of any one of claims 36-39, wherein the first cell population is a population of engineered $\gamma\delta$ T cells.~~

3045. The method of any one of claims ~~36-44~~27-29, wherein the method comprises genetically modifying the expanded first $\gamma\delta$ T cell population or genetically modifying the enriched $\gamma\delta$ T cell population.

3146. The method of any one of claims ~~36-45~~27-30, wherein the method comprises producing an enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta\gamma$ $\gamma\delta$ T cells.

~~47. The method of claim 46, optionally~~ wherein the enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta\gamma$ $\gamma\delta$ T cells is produced within 12 to 21 days; or the method comprises expanding the $\delta\gamma$ $\gamma\delta$ T cells in the first cell population at least 1,000-fold, o at least 5,000-fold, or at least, or about, or at least about, 10,000 fold, optionally wherein the specified-fold expansion is achieved within 12 to 21 days.

~~48. The method of any one of claims 36-47, wherein the method comprises expanding the $\delta\gamma$ $\gamma\delta$ T cells in the first cell population at least 1,000-fold, preferably at least 5,000-fold, more preferably at least, or about, or at least about, 10,000-fold.~~

3249. The method of any one of claims ~~27-31~~48, wherein

i) at least one of the one or more first activating agents is structurally identical to at least one of the one or more second activating agents;

ii) ~~51. The method of any one of claims 36-50, wherein~~ at least one of the one or more first activating agents is structurally different than at least one of the one or more second activating agents; and/or

iii) ~~52. The method of any one of claims 36-51, wherein~~ at least one of the one or more first activating agents or at least one of the one or more second activating agents is immobilized.

~~53. The method of claim 52, optionally~~ wherein the immobilized activating agent is immobilized on a surface of a culture vessel.

~~54. The method of claim 52, wherein the immobilized activating agent is immobilized or~~ on a surface of an antigen presenting cell (APC) or an artificial antigen presenting cell (aAPC).

~~55. The method of any one of claims 52-54,); optionally~~ wherein immobilized activating agent is the antibody or fragment thereof that binds the epitope specific to a $\delta 3$ $\gamma\delta$ TCR.

~~3356.~~ A method according to any one of claims ~~22-35 or 36-55~~13-30, wherein the method achieves ~~greater than 10%, greater than 20%, or~~ greater than 30% $\delta 3$ $\gamma\delta$ T cells prior to, or in the absence of, $\alpha\beta$ T cell depletion, preferably greater than 40%.~~57. An expanded $\gamma\delta$ T cell population, wherein greater than 30% of the expanded $\gamma\delta$ T cells are $\delta 3$ $\gamma\delta$ T cells, preferably greater than 40%, more preferably greater than 60%, yet more preferably greater than 70%, even more preferably greater than 80%.~~

34. An enriched $\gamma\delta$ T cell population produced by the method according to any one of claims 12-33.

35. The enriched $\gamma\delta$ T cell population of claim 34, wherein the enriched $\gamma\delta$ T cell population comprises greater than 10%, greater than 20%, or greater than 30% $\delta 3$ $\gamma\delta$ T cells.

~~3658.~~ The ~~enriched~~expanded $\gamma\delta$ T cell population of claim 34 or 35~~57~~, wherein the ~~expanded~~enriched $\gamma\delta$ T cell population comprises polyclonal TCR diversity.

~~59. The expanded $\gamma\delta$ T cell population of claim 58, optionally~~ wherein greater than 60% or 70% the $\delta 3$ T-cells express the phenotype CD45RA+/CD27+ and/or CD45RA-/CD27+.

3760. The ~~expanded~~enriched $\gamma\delta$ T cell population of any one of claims ~~57-59~~34-36, which is derived from tumor infiltrating lymphocytes.

3861. The ~~expanded~~enriched $\gamma\delta$ T cell population of any one of claims ~~57-60~~34-37, wherein the $\gamma\delta$ T-cells express an endogenous or heterologous tumor recognition moiety.

3962. The ~~expanded~~enriched $\gamma\delta$ T cell population of any one of claims ~~57-61~~34-38, wherein the population comprises a therapeutically effective amount of $\gamma\delta$ T-cells.

4063. The ~~expanded~~enriched $\gamma\delta$ T cell population of any one of claims ~~57-62~~34-39, wherein the enriched $\gamma\delta$ T cell population comprises anti-tumor cytotoxicity that is independent of NKp30 activity, NKp44 activity, and/or NKp46 activity.

~~64. The expanded $\gamma\delta$ T cell population of claim 63, optionally~~ wherein the $\gamma\delta$ T cell population does not comprise NKp30 activity-dependent anti-tumor cytotoxicity, NKp44 activity-dependent anti-tumor cytotoxicity, and/or NKp46 activity-dependent anti-tumor cytotoxicity; and/or wherein less than 40% of the $\gamma\delta$ T cells express a detectable level of NKp30, NKp44, and/or NKp46.

41. A composition comprising the enriched $\gamma\delta$ T cell population or any one of claims 34-40; and the antibody or fragment thereof of any one of claims 1-7.

~~65. The expanded $\gamma\delta$ T cell population of any one of claims 57-63, wherein less than 40% of the $\gamma\delta$ T cells express a detectable level of NKp30, NKp44, and/or NKp46.~~

~~66. An expanded $\gamma\delta$ T cell population, which is produced by:~~

~~a) a method according to any one of claims 22-35; or~~

~~b) a method according to any one of claims 36-56.~~

~~67. A method of treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof, the method comprising administering a therapeutically effective amount of $\delta\gamma$ T cells to the subject.~~

~~68. A method of treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof, the method comprising:~~

~~a) providing a therapeutically effective amount of $\delta\gamma$ T cells according to any one of claims 57-66; and~~

~~b) administering the therapeutically effective amount of $\delta\gamma$ T cells to the subject~~

42. Use of an enriched $\delta\gamma$ T cell population according to any one of claims 34-40 or the composition according to claim 41 in the manufacture of a medicament for treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof.

4369. The method-use of claim ~~68~~42, wherein the ~~method further comprises admixing the expanded~~enriched $\gamma\delta$ T cell population is admixed with a second expanded $\gamma\delta$ T cell population to form an admixed population ~~and administering the admixed population to the subject.~~

~~70. The method of claim 69, optionally~~ wherein:

i) the second expanded $\gamma\delta$ T cell population comprises $> 60\%$ $\delta 1$ or $> 60\%$ $\delta 2$ $\gamma\delta$ T cells;

~~71. The method of claim 69, wherein ii)~~ the admixed $\gamma\delta$ T cell population comprises $> 60\%$ $\delta 1$ or $> 60\%$ $\delta 2$ $\gamma\delta$ T cells; or

~~72. The method of claim 69, wherein iii)~~ the admixed $\gamma\delta$ T cell population comprises $> 60\%$ $\delta 3$ $\gamma\delta$ T cells.

44. An enriched $\delta 3$ $\gamma\delta$ T cell population according to any one of claims 34-40 or the composition according to claim 41 for use in therapy.