The ability of γδ T-cells to recognize an antigen on diseased cells directly and to exhibit inherent ability to kill tumor cells renders γδ T-cells an attractive therapeutic tool. Adoptive transfer of Vγ9Vδ2 T cells has yielded limited objective clinical responses for investigational treatment of cancer (Kondo et al, Cytotherapy, 10:842- 856, 2008; Lang et al, Cancer Immunology, Immunotherapy: CII, 60: 1447-1460, 2011; Nagamine et al , 2009; Nicol et al, British Journal of Cancer, 105:778-786, 2011; Wilhelm et al, Blood. 2003 Jul 1;102(1):200-6), indicating the need to isolate and test clinically new γδ T-cell populations.

The ability to selectively expand γδ T-cell subset populations having potent anti-tumor activity with improved purity and in clinically-relevant levels is highly desirable. Although antibodies and cytokine cocktails have been used to propagate a more diverse set of γδ T cells, activation of specific γδ T-cell subsets to sufficient purity and clinically-relevant levels, was not achieved (Dokouhaki et al, 2010; Kang et al, 2009; Lopez et al, 2000; Kress, 2006). Therefore, clinically-relevant methods of expanding specific γδ T cell subsets ex vivo, and the cells produced thereby, are greatly needed.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 21, 2017 is named ADC-0002-WO_SL.txt and is 207,179 Kilobytes in size.

SUMMARY OF THE INVENTION

The present invention, in one aspect, provides *ex vivo* methods for producing enriched γδ T-cell populations. The enriched γδ T-cell populations can be produced from isolated mixed cell populations by a method comprising contacting the mixed cell population, or a purified fraction thereof, with one or more agents:

i) which selectively expand δ1 T-cells by binding to an epitope specific of a δ1 TCR,

ii) which selectively expand δ2 T-cells by binding to an epitope specific of a δ2 TCR,

iii) which selectively expand δ1 and δ4 T-cells by binding to an epitope specific of a δ1 TCR and a δ4 TCR, or

FIG. 4 depicts a graph illustrating growth of γδ1 and γδ2 lymphocytes isolated from colon adenocarcinoma metastasis to liver (TIL 1) and renal tumors (TIL 2) and have been shown to express CCR4 and CCR7.

FIG. 5 depicts a graph illustrating γδ T-cells growth in serum-containing and serum-free media.

FIG. 6 depicts a graph illustrating anti-γδ TCR antibody blocking experiments with 5A6.E9, B1, TS8.2, 15D, B3, B6, TS-1, γ3.20, IMMU510, or 11F2.

FIG. 7 depicts a graph illustrating anti-γδ TCR antibody blocking experiments with 5A6.E9, B1, TS8.2, 15D, B3, B6, TS-1, γ3.20, IMMU510, or 11F2.

FIG. 8 depicts a competition experiment with anti-TCR Vδ1 TS-1 antibody.

FIG. 9 depicts a competition experiment with anti-TCR Vδ1 TS8.2 antibody.

FIG. 10 depicts a graph illustrating activation and expansion of δ1 T-cells from PBMC.

FIG. 11 depicts a graph illustrating activation and expansion of δ2 T-cells from PBMC.

FIG. 12 depicts a graph illustrating fold expansion of δ1 T-cells from PBMC.

FIG. 13 depicts a graph illustrating fold expansion of δ2 T-cells from PBMC.

FIG. 14 depicts the human Vδ1 amino acid sequence (SEQ ID NO: 133). CDR1, CDR3, and CDR3 regions are underlined. FIG. 14 also discloses the nucleotide sequence as SEQ ID NO: 132.

FIG. 15 depicts the human Vδ2 amino acid sequence (SEQ ID NO: 135). CDR1, CDR3, and CDR3 regions are underlined. FIG. 15 discloses SEQ ID NOS 135, 134, 137, and 136, respectively, in order of appearance.

FIG. 16 depicts activation of PBMCs with TS-1 or TS8.2 antibodies resulting in significant and specific expansion of $V\delta1^+$ T cells. **(A)** MAbs were directly coated (1 μg/mL) (TS1, TS8.2, B6, and 15D) or captured (0.1μg/mL) with goat-anti-mouse Fc (5 μg/mL) (TS1Fc, TS8.2Fc, B6Fc and 15DFc) in 24-well plates. PBMCs were plated at 10^6 cells/mL in RPMI with 10% FBS and 100 IU/mL IL-2. On day 7, cells were transferred to new plates without antibodies and further expanded until day 14. Culture media was replenished every 2-3 days. Data depicts expansion of $V\delta1^+$ cells over 14 days; **(B)** Same culture as A, showing percentage of $V\delta1^+$ cells on day 14 and day 0 (d0); **(C)** $V\delta1^+$ cells were expanded from isolated PBMCs from a different donor as follows. MAbs were directly coated (1 μg/mL) (TS1, TS8.2, B6 and 15D) or captured (0.1 μg/mL) with goat-anti-mouse Fc (5 μg/mL) (TS1Fc, TS8.2Fc, B6Fc and 15DFc) in 24-well plates. PBMCs f were plated at 10^6 cells/mL in RPMI with 10% FBS and 100 IU/mL IL-2. Cells were transferred to new plate without

to the Vδ1J1 chain at the selected positions. BE-13 refers to δ1J region from a T cell leukemia cell line expressing δ1γ8 TCR (DSMZ accession #ACC 396). Replacement of a single amino acid residue at position Lys120 by Thr or Ala in the Vδ1J1 and Vδ1J2 region completely abolished binding of TS-1 and TS8.2 MAbs indicating that this amino acid in the Vδ1J1 and Vδ1J2 region is contributes to the binding of TS-1 and TS8.2. Replacement of a single amino acid residue at position Thr120 by Lys in the Vδ1J3 region resulted in a gain of binding of TS-1 and TS8.2 MAbs further indicating that this amino acid in the Vδ1J1 and Vδ1J2 region contributes to the binding of TS-1 and TS8.2. FIG. 24 discloses SEQ ID NOS 138-143, respectively, in order of appearance.

FIG. 25 depicts that point mutation in Vδ1J1 from Lys120 to Thr or Ala resulted in the loss of binding by TS-1 and TS8.2 MAbs.

FIG. 26 depicts of human Vδ1 protein sequence (SEQ ID NO: 144) and the six mutated human Vδ1 sequences (SEQ ID NOS 145-150, respectively, in order of appearance) based on the differences between human Vδ1 and the bovine Vδ1 amino acid sequence (GenBank:AFP25162.1). Mutation are shown in bold.

FIG. 27 depicts loss of binding of TS-1 and TS8.2 antibodies to the human Vδ1 mutated chains.

FIG. 28 depicts binding of the B6 MAb to different Vδ2/γ chain pairings (γ3, γ8, γ9).

FIG. 29 depicts the protein sequence alignment of human (IMGT Human TRDV2) (SEQ ID NO: 151) and Rhesus monkey (GenBank: AY190028.1) (SEQ ID NO: 152) Vδ2 variable regions and the changes made in Vδ2 CDR1 (G35S), CDR2 (D65G) and CDR3 (C104S).

FIG. 30 depicts loss of binding of 15D to Vδ2 (G35S) mutated at CDR1.

FIG. 31 depicts characterization of highly enriched Vδ1+, Vδ2+ and αβ T cell cultures using TS8.2, B6 and IP26 antibodies for δ1, δ2 and αβ, respectively. The populations derived from PBMC were expanded with a combination of TS-1 and TS8.2 to expand δ1 cells, or 15D and B6 to expand δ2 cells. The expanded culture was depleted of αβT cells using IP26 MicroBeads. Positively selected αβ T cells were also collected (αβ enriched).

FIG. 32 depicts cytotoxicity of Vδ1+, Vδ2+ populations toward solid tumor (BxPC3, SKMEL5) and plasmacytoma (RPMI8226) cell lines.

FIG. 33 depicts heavy-chain framework and complementarity determining region amino acid sequences of δ1-specific MAbs. FIG. 33 discloses SEQ ID NOS 153-179, respectively, in order of appearance.

FIG. 34 depicts light-chain framework and complementarity determining region amino acid

sequences of the δ1-specific MAbs described in **FIG. 33**. FIG. 34 discloses SEQ ID NOS 180-206, respectively, in order of appearance.

FIG. 35 depicts heavy-chain framework and complementarity determining region amino acid sequences of δ2-specific MAbs. FIG. 35 discloses SEQ ID NOS 207-215, respectively, in order of appearance.

FIG. 36 depicts light-chain framework and complementarity determining region amino acid sequences of δ2-specific MAbs. FIG. 36 discloses SEQ ID NOS 216-225, respectively, in order of appearance.

FIG. 37 depicts cross-reactivity of the indicated δ1-specific antibodies with other γδ TCRs as determined by ELISA.

FIG. 38 depicts an alignment of Vδ1 deduced amino acid sequence between human (SEQ ID NO: 133) and dolphin (SEQ ID NO: 226).

FIG. 39 depicts the percentage of proliferating Vδ1 cells (A) or Vδ2 cells (B) obtained by a γδ T cell expansion method described herein.

FIG. 40 depicts the fold expansion of Vδ1 cells (A) or Vδ2 cells (B) from an isolated mixed cell population obtained by a γδ T cell expansion method described herein.

FIG. 41 depicts phenotype data from an isolated mixed cell population obtained by a γδ T cell expansion method described herein. Cell phenotypes are determined by CD45RA and CD27 expression using flow cytometry analysis.

FIG. 42 illustrates δ1 T cells activated and expanded according to γδ T cell expansion methods described herein can show robust cytotoxicity against tumor cell lines.

FIG. 43 illustrates Vδ1 fold expansion and purity on day 19 achieved by a method of the present invention.

FIG. 44 illustrates Vδ1 fold expansion and accumulative doubling time on day 19 achieved by a method of the present invention.

FIG. 45 illustrates Vδ1 fold expansion and accumulative doubling time from day 14 to day 19 achieved by a method of the present invention.

FIG. 46 illustrates expression of an anti-CD20 chimeric antigen receptor (CAR) on δ1 T cells. Expanded cells were stained with Vδ1 specific antibody (R9.12) and anti-Rituximab FITC conjugate on Day 7 post-transduction with CAR retroviral construct. Up to 35% of Vδ1 cells were CD20 CAR+.

FIG. 47A-B depicts epitope binding specificity data for δ-1 specific γδ T cell activators.

FIG. 48 depicts epitope binding specificity data for δ-2 specific γδ T cell activators.

determining spatial conformation of amino acids with methods that include, e.g., x-ray crystallography and 2-dimensional nuclear magnetic resonance. Some epitope mapping methods, such as, x-ray analyses of crystals of antigen:antibody complexes can provide atomic resolution of the epitope. In other cases, computational combinatorial methods for epitope mapping can be employed to model a potential epitope based on the sequence of the antibody, such as TS-1 antibody or TS8.2 antibody. In such cases, the antigen binding portion of the antibody is sequenced, and computation models are used to reconstruct and predict a potential binding site of the antibody.

In some cases the disclosure provides a method of determining an epitope of a γδ T-cell receptor, comprising: (a) preparing a library of epitopes from the γδ T-cell receptor; (b) contacting the library of epitopes with an antibody; and (b) identifying the amino acid sequence of at least one epitope in the library of epitopes that is bound by the antibody. In some cases, the antibody is selected from the group consisting of, TS8.2, 15D, B6, TS-1, and R9.12, antibodies. In one instance, the antibody is attached to a solid support. The library of epitopes can comprise sequences that correspond to continuous and discontinuous epitopes of a T-cell receptor, such as a γ TCR or a δ TCR. In some cases, the library of epitopes comprises fragments from a γδ T-cell receptor ranging from about 10 amino acids to about 30 amino acids in length, from about 10 amino acids to about 20 amino acids in length, or from about 5 amino acids to about 12 amino acids in length. In some cases, the antibody is labeled and the label is a radioactive molecule, a luminescent molecule, a fluorescent molecule, an enzyme, or biotin.

<u>δ1 Epitope Bins</u>

In some embodiments, the epitope, that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 47-70 of human Vδ1 (SKEMIFLIRQ GSDEQNA) (SEQ ID NO: 17) and J1 (TDKLIFGKGTRVTVEP) (SEQ ID NO: 18) or J2 (LTAQLFFGKGTQLIVEP) (SEQ ID NO: 19), wherein the activating agent does not bind an epitope containing a K120T mutation in J1 or J2. This epitope is referred to herein as a Bin 1 δ1 epitope. Exemplary activating agents that bind a Bin 1 δ1 epitope include but are not limited to TS-1; and δ1-18. A human Vδ1 J region can vary due to (D)(J) recombination, an exemplary Vδ1 J region of a δ1 chain of a γδ TCR having the Bin 1 δ1epitope is SKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQD TDKLIFGKGTRVTVEP (SEQ ID NO: 20); and another exemplary Vδ1 J region Bin 1 δ1epitope of

a δ1 chain of a γδ TCR is SKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGK HLTAQLFFGKGTQLIVEP (SEQ ID NO: 21).

In some cases, activating agents that bind a Bin 1 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDSWDTRQMFFGTGIK LFVEP (SEQ ID NO: 23).

The activating agents that bind a Bin 1 δ1 epitope can also bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGKHLTAQLFFGKGTQLI VEP (SEQ ID NO: 24).

In some embodiments, the epitope, that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 47-70 of human Vδ1 (SKEMIFLIRQ GSDEQNA) (SEQ ID NO: 17) and J1 (TDKLIFGKGTRVTVEP) (SEQ ID NO:18), wherein the activating agent does not bind an epitope containing a K120T mutation in J1. This epitope is referred to herein as a Bin 1b δ1 epitope. Exemplary activating agents that bind a Bin 1b δ1 epitope include but are not limited to δ1-37. An exemplary Vδ1 J region of a δ1 chain of a γδ TCR having the Bin 1b δ1epitope is SKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQD TDKLIFGKGTRVTVEP (SEQ ID NO: 20).

In some cases, activating agents that bind a Bin 1b δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDSWDTRQMFFGTGIK LFVEP (SEQ ID NO: 23).

The activating agents that bind a Bin 1b δ1 epitope also do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGKHLTAQLFFGKGTQLI VEP (SEQ ID NO: 24).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 11-21 of human Vδ1 (VSMPVRKAVTL) (SEQ ID NO: 26). This epitope is referred to herein as a Bin 2 δ1 epitope. Exemplary activating agents that bind a Bin 2 δ1 epitope include but are not limited to and δ1-285.

In some cases, activating agents that bind a Bin 2 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQVQRAMSSQLGEAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE PRSQPHTKPSVFVMKNGTNVACLVKEF (SEQ ID NO: 25).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 11-21 of human Vδ1 (VSMPVRKAVTL) (SEQ ID NO: 26), wherein the activating agent that binds this epitope does not bind an epitope containing a mutation of R16 in Vδ1, such as an R16N mutation. This epitope is referred to herein as a Bin 2b δ1 epitope. Exemplary activating agents that bind a Bin 2b δ1 epitope include but are not limited to R9.12.

In some cases, activating agents that bind a Bin 2b δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE

P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVNKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 27); and/or do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQVQRAMSSQLGEAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE PRSQPHTKPSVFVMKNGTNVACLVKEF (SEQ ID NO: 25).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 11-21 of human Vδ1 (VSMPVRKAVTL) (SEQ ID NO: 26), wherein the activating agent that binds this epitope also binds (cross-reacts) with δ3, δ4, and δ5 γδ TCRs. This epitope is referred to herein as a Bin 2c δ1 epitope. Exemplary activating agents that bind a Bin 2c δ1 epitope include but are not limited to δ1-39.

In some cases, activating agents that bind a Bin 2c δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQVQRAMSSQLGEAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE PRSQPHTKPSVFVMKNGTNVACLVKEF (SEQ ID NO: 25).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 80-95 of human Vδ1 (FKKAAKSVALTISALQ) (SEQ ID NO: 28) or 70 to 95 of human Vδ1 (AKSGRYSVNFKKAAKSVALTISALQ) (SEQ ID NO: 29). This epitope is referred to herein as a Bin 3 δ1 epitope. Exemplary activating agents that bind a Bin 3 δ1 epitope include but are not limited to δ1-08; and δ1-23.

In some cases, activating agents that bind a Bin 3 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE

P (SEQ ID NO: 22)

but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSGEMTFLIHQISSDQ NAKNGRYSVNFQERHKFISLTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVEP RSQPHTKPSVFVMKNGTNVACLVKEFYPKD (SEQ ID NO: 30).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 1-11 of human Vδ1 (AQKVTQAQSSV) (SEQ ID NO: 227) and J1 or J2. This epitope is referred to herein as a Bin 4 δ1 epitope. In some cases, the Bin 4 δ1 epitope binding activating agent does not bind an epitope containing a K120T mutation in J1 or J2. Exemplary activating agents that bind a Bin 4 δ1 epitope include but are not limited to δ1-35; and δ1-203.

In some cases, activating agents that bind a Bin 4 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDSWDTRQMFFGTGIK LFVEP (SEQ ID NO: 23).

In some cases, activating agents that bind a Bin 4 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGKHLTAQLFFGKGTQLI VEP (SEQ ID NO: 24).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 28-47 of human Vδ1 (SWWSYYIFWYKQLPS) (SEQ ID NO: 31) and J1. This epitope is referred to herein as a Bin 5 δ1 epitope. Exemplary activating agents that bind a Bin 5 δ1 epitope include but are not limited to δ1-113; δ1-155; δ1-183; δ1-191; δ1-278; and δ1-282. An exemplary Vδ1 J1 region of a δ1 chain of a γδ TCR having the Bin 5 δ1epitope is

SWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAK YFCALGTGVRGLQDTDKLIFGKGTRVTVEP (SEQ ID NO: 32).

In some cases, activating agents that bind a Bin 5 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGKHLTAQLFFGKGTQLI VEP (SEQ ID NO: 24).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 21-28 of human Vδ1 (LNCLYETS) (SEQ ID NO: 33) and J1. This epitope is referred to herein as a Bin 6 δ1 epitope. Exemplary activating agents that bind a Bin 6 δ1 epitope include but are not limited to TS 8.2 and δ1-143. An exemplary Vδ1 J1 region of a δ1 chain of a γδ TCR having the Bin 6 δ1epitope is LNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISAL QLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVEP (SEQ ID NO: 34).

In some cases, activating agents that bind a Bin 6 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDSWDTRQMFFGTGIK LFVEP (SEQ ID NO: 23).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 47-70 of human Vδ1 (SKEMIFLIRQGSDEQNA) (SEQ ID NO: 17) and J1 or J2. This epitope is referred to herein as a Bin 7 δ1 epitope. Exemplary activating agents that bind a Bin 7 δ1 epitope include but are not limited to δ1-149; δ1-253, and δ1-257. An exemplary Vδ1 J region of a δ1 chain of a γδ TCR

having the Bin 7 δ1epitope is SKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQD TDKLIFGKGTRVTVEP (SEQ ID NO: 20); and another exemplary Vδ1 J region Bin 7 δ1epitope of a δ1 chain of a γδ TCR is SKEMIFLIRQGSDEQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGK HLTAQLFFGKGTQLIVEP (SEQ ID NO: 21).

In some cases, activating agents that bind a Bin 7 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDSWDTRQMFFGTGIK LFVEP (SEQ ID NO: 23).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 70-80 of human Vδ1 (AKSGRYSVNF) (SEQ ID NO: 35) and J1 or J2. This epitope is referred to herein as a Bin 8 δ1 epitope. Exemplary activating agents that bind a Bin 8 δ1 epitope include but are not limited to δ1-192. An exemplary Vδ1 J region of a δ1 chain of a γδ TCR having the Bin 8 δ1epitope is AKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVEP (SEQ ID NO: 36); and another exemplary Vδ1 J region Bin 8 δ1epitope of a δ1 chain of a γδ TCR is AKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGEAPSAWGKHLTAQLFFGKGTQLIV EP (SEQ ID NO: 37).

In some cases, activating agents that bind a Bin 8 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDSWDTRQMFFGTGIK

LFVEP (SEQ ID NO: 23).

In some embodiments, the epitope that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 80-95 of human Vδ1 (FKKAAKSVALTISALQ) (SEQ ID NO: 28). This epitope is referred to herein as a Bin 9 δ1 epitope. Exemplary activating agents that bind a Bin 9 δ1 epitope include but are not limited to δ1-201.

In some cases, activating agents that bind a Bin 9 δ1 epitope bind a δ1 chain of a γδ TCR having the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVEP (SEQ ID NO: 22); but do not bind a δ1 chain of a γδ TCR having the sequence of

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSGEMTFLIHQISSDQ NAKNGRYSVNFQERHKFISLTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVEP (SEQ ID NO: 38).

The δ1-specific antibodies described herein selectively bind δ1-containing γδ-TCRs over δ2-containing γδ-TCRs. As such, the foregoing δ1-specific antibodies do not bind, *e.g.*, the sequence of and/or a γδ-TCR comprising the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP GFKDNFQGDIDIAKNLAVLKILAPSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 39).

<u>δ2 Epitope Bins</u>

In some embodiments, the epitope, that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 83-94 of human Vδ2 (AKNLAVLKILAP) (SEQ ID NO: 40). This epitope is referred to herein as a Bin 1 δ2 epitope. In some cases, the activating agent that binds the Bin 1 δ2 epitope does not bind an epitope containing a mutation of K90 in Vδ2, such as an K90N mutation. Exemplary activating agents that bind a Bin 1 δ2 epitope include but are not limited to δ2-17, and B6.

In some cases, activating agents that bind a Bin 1 δ2 epitope bind a δ2 chain of a γδ TCR having the sequence of

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP

GFKDNFQGDIDIAKNLAVLKILAPSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 39); but do not bind a δ2 chain of a γδ TCR having the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP GFKDNFQGDIDFLNNQAVLNILEASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 41).

In some embodiments, the epitope, that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 28-38 of human Vδ2 (EAIGNYY) (SEQ ID NO: 42). This epitope is referred to herein as a Bin 2 δ2 epitope. In some cases, the activating agent that binds the Bin 2 δ2 epitope does not bind an epitope containing a mutation of G35 in Vδ2, such as an G35S mutation. Exemplary activating agents that bind a Bin 2 δ2 epitope include but are not limited to 15D.

In some cases, activating agents that bind a Bin 2 δ2 epitope bind a δ2 chain of a γδ TCR having the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP GFK.DNFQGDIDIAKNLAVLKILAPSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 39); but do not bind a δ2 chain of a γδ TCR having the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGDSISNYYTFWYRRTPGNTMTLIYREGGTYG PGFEDNLQGEIDFLNNQAVLNILEASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 43).

In some embodiments, the epitope, that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 72-83 of human Vδ2 (KDNFQGDIDIA) (SEQ ID NO: 44). This epitope is referred to herein as a Bin 3 δ2 epitope. Exemplary activating agents that bind a Bin 3 δ2 epitope include but are not limited to δ2-32.

In some cases, activating agents that bind a Bin 3 δ2 epitope bind a δ2 chain of a γδ TCR having the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP GFK.DNFQGDIDIAKNLAVLKILAPSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 39); but do not bind a δ2 chain of a γδ TCR having the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP GFEDNLQGEIDFLNNQAVLNILEASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ

ID NO: 45).

In some embodiments, the epitope, that is (*e.g.*, specifically) recognized by an activating agent of interest is an epitope comprised of amino acids 1-27 of human Vδ2 (AIELVPEHQTVPVSIGVPATLRCSMKG) (SEQ ID NO: 46). This epitope is referred to herein as a Bin 4 δ2 epitope. Exemplary activating agents that bind a Bin 4 δ2 epitope include but are not limited to δ2-14; δ2-22; δ2-30; δ2-31; δ2-36; and δ2-37.

In some cases, activating agents that bind a Bin 4 δ2 epitope bind a δ2 chain of a γδ TCR having the sequence of:

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGP GFKDNFQGDIDIAKNLAVLKILAPSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 39); but do not bind a δ2 chain of a γδ TCR having the sequence of:

AVTLVPQNQARSVSVGESVTLRCSMKGDSISNYYTFWYRRTPGNTMTLIYREGGTY GPGFEDNLQGEIDFLNNQAVLNILEASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 47).

The δ2-specific antibodies described herein selectively bind δ2-containing γδ-TCRs over δ1-containing γδ-TCRs. As such, the foregoing δ2-specific antibodies do not bind, *e.g.*, the sequence of and/or a γδ-TCR comprising the sequence of:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22).

Generally, the δ1- and δ2-specific antibodies described herein recognize a conformational epitope in the context of a γδ-TCR. In some cases, the δ1- and δ2-specific antibodies described herein are specific for one or more pairs of γδ1- or γδ2- TCRs respectively. For example, in some cases, the δ1- specific antibodies described herein are specific for a γ8δ1-TCR. In some cases, the δ1- specific antibodies described herein bind γ8δ1-TCR but not γ9δ1-TCR.

Methods of Treatment

Pharmaceutical compositions containing a non-engineered, enriched γδ T-cell population, an engineered, enriched γδ T-cell population, and/or admixtures thereof, as described herein may be administered for prophylactic and/or therapeutic treatments. In therapeutic applications, the

compositions can be administered to a subject already suffering from a disease or condition in an amount sufficient to cure or at least partially arrest the symptoms of the disease or condition. A non-engineered, enriched γδ T-cell population, an engineered, enriched γδ T-cell population, and/or admixtures thereof, can also be administered to lessen a likelihood of developing, contracting, or worsening a condition. Effective amounts of a population of a non-engineered, enriched γδ T-cell population, an engineered, enriched γδ T-cell population, and/or admixtures thereof, for therapeutic use can vary based on the severity and course of the disease or condition, previous therapy, the subject's health status, weight, and/or response to the drugs, and/or the judgment of the treating physician.

A non-engineered, enriched γδ T-cell population, an engineered, enriched γδ T-cell population, and /or admixtures thereof, of the disclosure can be used to treat a subject in need of

TCR chains were amplified and cloned out of TS8.2 and B6 activated populations. PBMCs cultures were harvested after 14 days of specific activation with δ1 specific activating mAbs TS8.2 and the δ2 specific MAb B6. PBMCs cultures were lysed in 350 µl RLT buffer (Qiagen,), Total RNA was purified from $5X10^5$ cell using the Qiagen RNeasy Mini Kit (Qiagen, catalog number 74106), following the manufacturer's instructions. In brief, 350 µl of 70% Ethanol was added to the cell lysate to provide ideal binding conditions. The lysate was then loaded onto the RNeasy silica membrane spin column. Contaminants were washed away. Concentrated RNA was eluted in 50 µl water.

One step RT PCR: Full length delta chains were amplified using the 5′ δ1 (5' GCCCAGAAGGTTACTCAAGCCCAGTC3') (SEQ ID NO: 48) and 5' δ2 (5' GCCATTGAGTTGGTGCCTGAACACC 3') (SEQ ID NO: 49) primers, in combination with 3′ human Cδ (5' TTACAAGAAAAATAACTTGGCAGTCAAGAGAAA 3') (SEQ ID NO: 50) primer. A 800 bp PCR fragments of the full length delta chain were cloned into the pCI expression vector (Promega catalog number E1841). Similarly, full length gamma chains were amplified using the 5' primers γ2,γ3,γ4 (5' TCTTCCAACTTGGAAGGGAGAACGAAGTC 3') (SEQ ID NO: 51) γ5 (5' TCTTCCAACTTGGAAGGGGGAACGA 3') (SEQ ID NO: 52) , γ8 (5' TCTTCCAACTTGGAAGGGAGAACAAAGTC 3') (SEQ ID NO: 53) γ9 (5' GCAGGTCACCTAGAGCAACCTCAAATTTCC 3') (SEQ ID NO: 54) in combination with a single 3' Cgamma primer (5' GGAAGAAAAATAGTGGGCTTGGGGGAA 3') (SEQ ID NO: 13)

The delta and gamma transcripts were amplified from 100 ng total RNA using reverse transcriptase polymerase chain reaction (RT-PCR). A total of five RT-PCR reactions were run for each sample activated with the TS8.2 MAb (δ1, γ2-γ4, γ5,γ8 and γ9) and two RT-PCR reactions were run for each sample activated with the B6 MAb (δ2, and γ9). The QIAGEN One Step RT-PCR kit was used for amplification, (Qiagen, Inc.). This kit provides a blend of Sensiscript and Omniscript Reverse Transcriptases, HotStarTaq DNA Polymerase, dNTP mix, buffer and Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates. Reaction mixtures were prepared that included 5µl of RNA, 0.5 of 100 µM of either delta or gamma chains primers (custom synthesized by IDT), 5 µl of 5×RT-PCR buffer, 1 µl dNTPs, 1 µl of enzyme

mix containing reverse transcriptase and DNA polymerase, and 0.4 µl of ribonuclease inhibitor RNasin (1 unit). The reaction mixture contains all of the reagents required for both reverse transcription and PCR. The thermal cycler program was RT step 50° C. for 30 minutes 95° C. for 15 minutes followed by 30 cycles of (95° C. for 30 seconds, 48° C. for 30 seconds, 72° C. for 1.0 minutes). There was then a final incubation at 72° C. for 10 minutes. The extracted PCR products were directly cloned into pCI expression vector. Nucleotide sequences were analyzed using IMGT to identify germline V, D and J gene members with the highest sequence homology.

Examples of sequences of Vδ1chains activated by TS8.2 CDR3 (junctional diversity is indicated by bold text):

TS8.2 activated clone1 Vδ1, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGPVVIPKGKLSF**GKGTRVTVEP (SEQ ID NO: 55)

TS8.2 activated clone2 Vδ1, D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGELCLGDTYTDKLIF**GKGTRVTVEP (SEQ ID NO: 56)

TS8.82 activated clone 3 Vδ1, D2+D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGDPKVYWGCTDKLIF**GKGTRVTVEP (SEQ ID NO: 57)

TS8.2 activated clone 4 Vδ1, D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQDAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGTGVRGLQDADKLIF**GKGTRVTVEP (SEQ ID NO: 58)

TS8.2 activated clone 5 Vδ1 D1+D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALLLGDTSFYTDKLIF**GKGTRVTVEP (SEQ ID NO: 59)

TS8.2 activated clone 6 Vδ1 D1+D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CAALLPFLPSDWGIPVTDKLIF**GKGTRVTVE P (SEQ ID NO: 60)

TS8.2 activated clone 7 Vδ1, D2 and D3, J1

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGTGVRGLQDTDKLIF**GTGTRVTVEP (SEQ ID NO: 61)

TS8.2 activated clone 8 Vδ1, D2 and D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGGLSSLDLGDTDNHYTDKLIF**GKGTRV TVEP (SEQ ID NO: 62)

TS8.2 activated clone 9 Vδ1, D2 and D3, J1
AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS GRYSVNFKKAAKSVALTISALQLEDSAKYF**CALGHPRSLMGVYTDKLIF**GKGTRVTVEP (SEQ ID NO: 63)

Examples of Gamma chains activated by TS8.2 Mab:

B8 TS8.82 activated Clone-1 γ4, J1/J2
SSNLEGRTKSVIRQTGSSAEITCDLAEGSTGYIHWYLHQEGKAPQRLLYYDSYTSSVVLESGI SPGKYDTYGSTRKNLRIILRNLIENDSGVYY**CATWDDGKKLF**GSGTTLVVT (SEQ ID NO: 64)

B8 TS8.82 activated Clone-2 γ9, JP1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWDDTRLGKKIKVF**APGTKLIIT (SEQ ID NO: 65)

TS8.2 activated Clone-3 γ4, J1
SSNLEGRTKSVIRQTGSSAEITCDLAEGSTGYIHWYLHQEGKAPQRLLYYDSYTSSVVLESGI SPGKYDTYGSTRKNLRMILRNLIENDSGVYY**CATWSDKKLF**GSGTTLVVT (SEQ ID NO: 66)

TS8.2 activated Clone-4 γ9, J1/J2
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWIHKKLF**GSGTTLVVT (SEQ ID NO: 67)

TS8.2 activated Clone 5 γ2, J1
SSNLEGRTKSVIRQTGSSAENTCDLAEGSNGYIHWYLHQEGKTPQRLQYYDSYNSKVVLES GVSPGKYYTYASTRNNLRLILRNLIENDSGVYY**CATWDCHYKKLF**GSGTTLVVT (SEQ ID NO: 68)

TS8.2 activated Clone 6 γ3,JP2
SSNLEGRTKSVTRQTGSSAEITCDLTVTNTFYIHWYLHQEGKAPQRLLYYDVSTARDVLESG LSPGKYYTHTPRRWSWILRLQNLIENDSGVYY**CATWDRRWIKTF**AKGTKLIVTSP (SEQ ID NO: 69)

TS8.2 activated Clone 7 γ2, JP1
SSNLEGRTKSVIRQTGSSAEITCDLAEGSNGYIHWYLHQEGKAPQRLQYYDSYNSKVVLES
GVSPGKYYTYASTRNNLRLILRNLIENDSGVYY**CATWDGLDATCGVDTTGWF**KIFAEGT
KLIVTSP (SEQ ID NO: 70)

TS8.2 activated Clone 8 γ4, J1/J2
SSNLEGRTKSVTRPTGSSAVITCDLPVENAVYTHWYLHQEGKAPRRLLYYDSYTSSVVLES
GISPGKYDTYGSTRKNLRMILRNLIENDSGVYY**CATKKLF**GSGTTLVVT (SEQ ID NO: 71)

TS8.2 activated Clone 9 γ9, JP1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI
PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEIASQLGKKIKVF**GPGTKLIIT (SEQ
ID NO: 72)

TS8.2 activated Clone 10 γ3, J1
SSNLGGRTKSVTRQTGSSAEITCDLTVTNTFYIHWYLHQEGKAPQRLLYYDVSTARDVLES
GLSPGKYYTHTPRRWSWILRLQNLIENDSGVYY**CATWDRYYYKKLF**GSGTTLVVT (SEQ
ID NO: 73)

TS8.2 activated Clone 11 γ3, JP2
SSNLEGRTKSVTRQTGSSAEITCDLTVTNTFYIHWYLHQEGKAPQRLLYYDVSTARDVLESG
LSPGKYYTHTPRRWSWILRLQNLIENDSGVYY**PNSSDWIKTFAK**GTKLIVTSP (SEQ ID NO:
74)

TS8.2 activated Clone 12 γ4,J2
SSNLEGGTKSVTRPTRSSAEITCDLAERNTFYIHWYLHQEGKAPQRLQYYDSYTSSVVLESG
ISPGKYDTYGSTRKNLRMILRNLIENDSGVYY**CATRDVPNYYKKLF**GSGTTLVVT (SEQ ID
NO: 75)

TS8.2 activated Clone 13 γ2, JP1
SSNLEGRTKSVIRQTGSSAEITCDLAEGSNGYIHWYLHQEGKAPQRLQYYDSYNSKVVLES
GVSPGKYYTYASTRNNLRLILRNLIENDSGVYY**CATWDGRVSYTTGWFKIF**AEGTKLIVTS
P (SEQ ID NO: 76)

TS8.2 activated Clone 14 γ4, J1/J2
SSNLEGRTKSVIRQTGSSAEITCDLAEGSTGYIHWYLHQEGKAPQRLLYYDSYTSSVVLESGI
SPGKYDTYGSTRKNLRMILRNLIENDSGVYY**CATWDKGRKLF**GSGTTLVVT (SEQ ID NO:
77)

TS8.2 activated Clone 15 γ9, J1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI
PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWETHYKKLF**GSGTTLVVT (SEQ ID NO:
78)

TS8.2 activated Clone 16 γ2, J1

SSNLEGRTKSVIRQTGSSAEITCDLAEGSNGYIHWYLHQEGKAPQRLQYYDSYNSKVVLES
GVSPGKYYTYASTRNNLRLILRNLIENDSGVYY**CATWDGRYKKLF**GSGTTLVVT (SEQ ID
NO: 79)

TS8.2 activated Clone 17 γ4, JP1
SSNLEGRTKSVIRQTGSSAEITCDLAEGSTGYIHWYLHQEGKAPQRLLYYDSYTSSVVLESGI
SPGKYDTYGSTRKNLRMILRNLIENDSGVYY**CATWGTTGWFKIF**AEGTKLIVTSP (SEQ ID
NO: 80)

B8(Buffy coat 8)- B6 activated clones:

B6 activated Clone δ2, D3,J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDKVLGVPTASYTDNKLIF**GKGTRVTVEP
(SEQ ID NO: 81)

B6 activated Clone δ2, D3,J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMSFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDTVGILPYDKLIF**GKGTRVTVEP (SEQ ID
NO: 82)

B6 activated Clone δ2, D2+D3, J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDILTVLGDNRTDKLIF**GKGTRVTVEP (SEQ
ID NO: 83)

B6 activated Clone δ2, D3,J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDVVGEGGADKLIF**GKGTRVTVEP (SEQ ID
NO: 84)

B6 activated Clone δ2, D3,J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDTVGGGEYTDKLIF**GKGTRVTVEP (SEQ
ID NO: 85)

B6 activated Clone δ2, D3,J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDTVGTGDIRTYTDKLIF**GKGTRVTVEP
(SEQ ID NO: 86)

B6 activated Clone δ2, D2+D3,J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD
NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDSLTGGSGLTDKLIF**GKGTRVTVEP (SEQ
ID NO: 87)

B6 activated Clone δ2, D3,J3
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD NFQGDIDIAKNLAVLKILPPSERDEGSYY**CACDTGGYSSWDTRQMF**FGTGIKLFVEP (SEQ ID NO: 88)

B6 activated Clone δ2, D2+D3, J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDPLKTLGTYTDKLIF**GKGTRVTVEP (SEQ ID NO: 89)

B6 activated Clone δ2, D1+D3, J1
AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKD NFQGDIDIAKNLAVLKILAPSERDEGSYY**CACDAVIAGGSFTDKLIF**GKGTRVTVEP (SEQ ID NO: 90)

B6 activated Gamma chains:

B6 activated clone γ9, JP1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEDQELGKKIKVF**GPGTKLIIT (SEQ ID NO: 91)

B6 activated clone γ9, JP1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWAYPPELGKKIKVF**GPGTKLIIT (SEQ ID NO: 92)

B6 activated clone γ9, JP1
AGHLEQPQISSTKTLSKTARLECAVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEVQELGKKIKVF**GPGTKLIIT (SEQ ID NO: 93)

B6 activated clone γ9, JP1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVCWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEVLQELGKKIKVF**GPGTKLIIT (SEQ ID NO: 94)

B6 activated clone γ9, JP1
AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVKKQDIATYY**CALWEVRELGKKIKVF**GPGTKLIIT (SEQ ID NO: 95)

B6 activated clone γ9,
JP1AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKE

SGIPSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWRELGKKIKVF**GPGTKLIIT (SEQ ID NO: 96)

B6 activated clone γ9, JP1AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKE SGIPSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEAQELGKKIKVF**GPGTKLIIT (SEQ ID NO: 97)

B6 activated clone γ9, JP1AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKE SGIPSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWETELGKKIKVF**GPGTKLIIT (SEQ ID NO: 98)

B6 activated clone γ9, JP1AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKE SGIPSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEEVELGKKIKVF**GPGTKLIIT (SEQ ID NO: 99)

B6 activated clone γ9, JP1 AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFPVSISYDGTVRKESGI PSGKFEVDRIPETSTSTLTIHNVEKQDIATYY**CALWEPPQSLGKKIKVF**GPGTKLIIT (SEQ ID NO: 100)

Example 34. Cryopreservation of γδ T-Cells in Freeze Media to Generate Cell Banks for Further Processing

Non-modified γδ T-Cells will be formulated in freezing media and placed in cryogenic storage units such as liquid nitrogen freezers (-195^0 C) or ultra-low temperature freezers (-65^0 C, -80^0 C or -120^0 C) for long-term storage. The freeze media will contain dimethyl sulfoxide (DMSO), sodium chloride (NaCl), dextrose, dextran sulfate or hydroyethyl starch (HES) with physiological pH buffering agents ranging between 6.5 and 7.5.

The cryopreserved γδ T-cells will be thawed and further processed by stimulation with antibodies, proteins, peptides, and cytokines. The cryopreserved γδ T-Cells will be thawed and genetically modified as previously described in this application. The engineered γδ T-cells will be further cryopreserved to generate cell banks in quantities of 10, 100, 200 vials at 10^6 to 10^8 cells per mL in freeze media. The non-engineered and/or engineered γδ T-cells will be further cryopreserved to generate cell banks in quantities of 10, 100, 200, 500, and 1,000 vials or bags at 10^6 to 10^8 cells per mL in freeze media.

HEK-293 cells were cultured in 90-mm cell culture plates in a DMEM medium (Thermo Fisher Scientific) with 10% fetal calf serum (HyClone™ FetalClone™ I Serum (U.S.) - GE Healthcare Life Sciences). For every construct 1×10^6 HEK-293 cells were transfected with 10 µg of plasmid using 293 Fectin as transfection reagent. Supernatants were harvested 72 hours post transfection

δ1 specific activators- monoclonal antibodies differed in their binding pattern to the chimeras and a distinct binding pattern could be identified for each monoclonal antibody, as tested in ELISA assay.

Polystyrene microtiter plates (Greiner bio-one # 655061) were coated overnight at room temperature with 1 µg/well of Goat anti human FC specific antibody (Jackson ImmunoResearch # 109-005-098) in ELISA coating buffer (50 mm Na2CO3, 0·05% NaH3, pH 9·6). The plates were washed three times with washing buffer (PBS 0·05% (v/v) Tween 20) and then blocked for 1 h at room temperature (RT) with 100 µl/well of ELISA blocking buffer (3%BSA in PBST). Binding of soluble TCRs to blocked plates was done by adding 100ml of each supernatant and incubation for 1 hour at Room temperature. Each antibody was diluted to 1 µg/ml in ELISA blocking buffer, and tested for binding to each γδ TCR chimeric molecule.

Detection of binding was done by adding 100 µl/well of HRP conjugated secondary antibody: Goat anti mouse FC specific (Jackson ImmunoResearch Laboratories, Inc. Peroxidase-AffiniPure Goat Anti-Mouse IgG, Fcγ Fragment Specific # 115-035-008) diluted to 1:10,000 in blocking buffer. The amount of bound antibodies was detected by the use of TMB substrate (Thermo Fisher #34029)Color development was measured spectrophotometrically at 450nm after 10 minutes. All assays were done in duplicate.

Sequence of all constructs

δ1-D3-J1 FC- Fully human TCR

gcccagaaggttactcaagcccagtcatcagtatccatgccagtgaggaaagcagtcaccctgaactgcctgtatgaaacaagttggt ggtcatattatatttttggtacaagcaacttcccagcaaagagatgattttccttattcgccagggttctgatgaacagaatgcaaaaagtggtcgcta ttctgtcaacttcaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacgg gggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 101)

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 22)

Dolphin TRD isolate 5R1D8

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgagctgtcagtatgaaacaagcttg agctggtacgatatttttttggtataagcagcttcccagtggagagatgactttccttattcatcagatttcttctgaccaaaatgcaaagaatggccgct attctgtaaactttcaggaaagacataaattcatcagcctcaccatttcagccttactggtggaagattctgcaaactacttctgtgctctccgggagc gcgttgtccacgtcgttttattcaatacgcgcaataagccactgctattcggcaaaggaacctatctgaacgttgaaccaa (SEQ ID NO: 102)

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSGEMTFLIHQISSDQ NAKNGRYSVNFQERHKFISLTISALLVEDSANYFCALRERVVHVVLFNTRNKPLLFGKGTYL NVEP (SEQ ID NO: 103)

Set of dolphin/human chimera -soluble TCRs were cloned -Human sequence in blue

Dolphin/Human 1-11

gcccagaaagttactcaagtccagcgagccatgtccatgccagtgaggaaagcagtcaccctgaactgcctgtatgaaacaagttggt ggtcatattatatttttttggtacaagcaacttcccagcaaagagatgattttccttattcgccagggttctgatgaacagaatgcaaaaagtggtcgcta ttctgtcaacttcaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacgg gggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 104)

AQKVTQVQRAMSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDE QNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTV EP (SEQ ID NO: 105)

Dolphin/Human 1-21

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgaactgcctgtatgaaacaagttggt ggtcatattatatttttttggtacaagcaacttcccagcaaagagatgattttccttattcgccagggttctgatgaacagaatgcaaaaagtggtcgcta

ttctgtcaacttcaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacgg gggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 106)

AQKVTQVQRAMSSQLGEAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 107)

Dolphin/Human 1-28

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgagctgtcagtatgaaacaagctgg tggtcatattatatttttttggtacaagcaacttcccagcaaagagatgattttccttattcgccagggttctgatgaacagaatgcaaaaagtggtcgct attctgtcaacttcaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacg ggggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 108)

AQKVTQVQRAMSSQLGEAVTLSCQYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 109)

Dolphin/Human 1-47

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgagctgtcagtatgaaacaagcttg agctggtacgatatttttttggtataagcagcttcccagtaaagagatgattttccttattcgccagggttctgatgaacagaatgcaaaaagtggtcgc tattctgtcaacttcaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacg ggggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 110)

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSKEMIFLIRQGSDEQ NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE P (SEQ ID NO: 111)

Dolphin/Human 1-70

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgagctgtcagtatgaaacaagcttg agctggtacgatatttttttggtataagcagcttcccagtggagagatgactttccttattcatcagatttctgatgaacagaatgcaaaaagtggtcgct

attctgtcaacttcaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacg
ggggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 112)

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSGEMTFLIHQISDEQ
NAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE
P (SEQ ID NO: 113)

Dolphin/human 1-80

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgagctgtcagtatgaaacaagcttg
agctggtacgatatttttttggtataagcagcttcccagtggagagatgactttccttattcatcagatttcttctgaccaaaatgcaaagaatggccgct
attctgtaaactttaagaaagcagcgaaatccgtcgccttaaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacgg
gggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 114)

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSGEMTFLIHQISSDQ
NAKNGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVE
P (SEQ ID NO: 115)

Dolphin/human 1-95

gcccagaaagttactcaagtccagcgagccatgtccagtcagctaggggaggcggtcaccttgagctgtcagtatgaaacaagcttg
agctggtacgatatttttttggtataagcagcttcccagtggagagatgactttccttattcatcagatttcttctgaccaaaatgcaaagaatggccgct
attctgtaaactttcaggaaagacataaattcatcagcctcaccatttcagccttacagctagaagattcagcaaagtacttttgtgctcttgggacgg
gggtgaggggactccaggacaccgataaactcatctttggaaaaggaacccgtgtgactgtggaaccaa (SEQ ID NO: 116)

AQKVTQVQRAMSSQLGEAVTLSCQYETSLSWYDIFWYKQLPSGEMTFLIHQISSDQ
NAKNGRYSVNFQERHKFISLTISALQLEDSAKYFCALGTGVRGLQDTDKLIFGKGTRVTVEP
(SEQ ID NO: 38)

RESULTS

Functional TCR genes are assembled from separate V, D and J region segments by genetic recombination. When the TCR delta chain gene is assembled a D gene segment is juxtaposed to a J segment and this is followed by rearrangement of a V segment to the assembled downstream DJ segment. The immunoglobulin-like fold of the TCR chains positions three loops or complementarity

Bin6: (aa 21-28+ J1 region): TS8.2; δ1-143

Bin7: (aa 47-70+ J1/J2 region): δ1-149; δ1-253, and δ1-257

Bin8: (aa 70-80) + J1/J2: δ1-192

Bin9: (aa 80-95): δ1-201.

Similar to the d1 epitope mapping: based on Linguiti G. et al., 2016. Genomic and expression analyses of Tursiops truncatus T cell receptor gamma (TRG) and alpha/delta (TRA/TRD) loci reveal a similar basic public; repertoire in dolphin and human. BMC Genomics. A set of 5 recombinant chimeric δ2 chains were cloned to produce human/dolphin δ2-FC TCR chains. Dolphin residues comprised amino acids 28-94, 44-94, 72-94 and, 82-94 respectively, according to IMGT unique numbering for T cell receptor variable domains (Lefranc et al., Developmental and Comparative Immunology 27 (2003) 55–77), as illustrated in **Fig.15.**

Transfection of δ2/γ9-FC constructs

Human TRGV9-JP-FC

GCAGGTCACCTAGAGCAACCTCAAATTTCCAGTACTAAAACGCTGTCAAAAACAGCCCGCCTGGAATGTGTGGTGTCTGGAATAACAAT
TTCTGCAACATCTGTATATTGGTATCGAGAGAGACCTGGTGAAGTCATACAGTTCCTGGTGTCCATTTCATATGACGGCACTGTCAGAA
AGGAATCCGGCATTCCGTCAGGCAAATTTGAGGTGGATAGGATACCTGAAACGTCTACATCCACTCTCACCATTCACAATGTAGAGAAA
CAGGACATAGCTACCTACTACTGTGCCTTGTGGGAGGTAAAGCAAGAGTTGGGCAAAAAAAATCAAGGTATTTGGTCCCGGAACAAAGCT
TATCATTACAG (SEQ ID NO: 117)

AGHLEQPQISSTKTLSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGIPSGKFEVDRIPETSTSTLTIHNVEK
QDIATYYCALWEVKQELGKKIKVFGPGTKLIIT (SEQ ID NO: 118)

Human Vδ21J1-FC

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATGACTTTCATATACCGAGAAAAGG
ACATCTATGGCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTT
GCACCATCAGAGAGAGATGAAGGGTCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 119)

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKDNFQGDIDIAKNLAVLKIL
APSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 39)

Dolphin Vδ2J1-FC

GCTGTCACGTTGGTGCCTCAAAACCAAGCAAGGAGTGTGTCTGTGGGGGAATCTGTCACCCTCAGGTGCTCCATGAAAGG
AGACTCCATCAGTAACTATTATACCTTCTGGTACAGGAGAACACCGGGTAACACAATGACTCTCATATACCGAGAAGGGG
GCACATATGGCCCTGGTTTCGAAGACAACCTCCAAGGTGAAATTGATTTTTTAAACAACCAGGCTGTGCTGAATATCCTG
GAGGCATCAGAGAGAGATGAAGGATCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAA
GGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 120)

AVTLVPQNQARSVSVGESVTLRCSMKGDSISNYYTFWYRRTPGNTMTLIYREGGTYGPGFEDNLQGEIDFLNNQAVLNIL
EASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 47)

Human/Dolphin Vδ2J1-FC (28-94)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGACTCCATCAGTAACTATTATACCTTCTGGTACAGGAGAACACCGGGTAACACAATGACTCTCATATACCGAGAAGGGG
GCACATATGGCCCTGGTTTCGAAGACAACCTCCAAGGTGAAATTGATTTTTTAAACAACCAGGCTGTGCTGAATATCCTG
GAGGCATCAGAGAGAGATGAAGGATCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAA
GGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 121)

AIELVPEHQTVPVSIGVPATLRCSMKGDSISNYYTFWYRRTPGNTMTLIYREGGTYGPGFEDNLQGEIDFLNNQAVLNIL
EASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 43)

Human/Dolphin Vδ2J1-FC (44-94)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAGAACACCGGGTAACACAATGACTCTCATATACCGAGAAGGGG
GCACATATGGCCCTGGTTTCGAAGACAACCTCCAAGGTGAAATTGATTTTTTAAACAACCAGGCTGTGCTGAATATCCTG
GAGGCATCAGAGAGAGATGAAGGATCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 122)

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRRTPGNTMTLIYREGGTYGPGFEDNLQGEIDFLNNQAVLNIL
EASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 123)

Human/ Dolphin Vδ2J1-FC (72-94)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATGACTTTCATATACCGAGAAAAGG
ACATCTATGGCCCTGGTTTCGAAGACAACCTCCAAGGTGAAATTGATTTTTTAAACAACCAGGCTGTGCTGAATATCCTG
GAGGCATCAGAGAGAGATGAAGGATCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 124)

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFEDNLQGEIDFLNNQAVLNIL
EASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 45)

Human Dolphin Vδ2J1-FC (82-94)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATGACTTTCATATACCGAGAAAAGG
ACATCTATGGCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATTTTTTAAACAACCAGGCTGTGCTGAATATCCTG
GAGGCATCAGAGAGAGATGAAGGATCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 125)

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKDNFQGDIDFLNNQAVLNIL
EASERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 41)

Human Vδ21J1-FC (CDR1-S)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCAGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATGACTTTCATATACCGAGAAAAGG
ACATCTATGGCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTT
GCACCATCAGAGAGAGATGAAGGGTCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 126)

AIELVPEHQTVPVSIGVPATLRCSMKGEAISNYYINWYRKTQGNTMTFIYREKDIYGPGFKDNFQGDIDIAKNLAVLKIL
APSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 127)

Human Vδ21J1-FC (CDR2-G)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATGACTTTCATATACCGAGAAAAGG
GCATCTATGGCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTT
GCACCATCAGAGAGAGATGAAGGGTCTTACTACTGTGCCTGTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 128)

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKGIYGPGFKDNFQGDIDIAKNLAVLKIL
APSERDEGSYYCACDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 129)

Human Vδ21J1-FC (CDR3-S)

GCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGCTCCATGAAAGG
AGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATGACTTTCATATACCGAGAAAAGG
ACATCTATGGCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTT
GCACCATCAGAGAGAGATGAAGGGTCTTACTACTGTGCCTCTGACCCTCTTGGCGGACCCCCCGATAAACTCATCTTTGG
AAAAGGAACCCGTGTGACTGTGGAACCAA (SEQ ID NO: 130)

AIELVPEHQTVPVSIGVPATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDIYGPGFKDNFQGDIDIAKNLAVLKIL
APSERDEGSYYCASDPLGGPPDKLIFGKGTRVTVEP (SEQ ID NO: 131)

ELISA results are shown in **Fig. 48,** identifying the following epitope bins:

Bin	1	2	3	4
Residues	83-94	28-38	72-83	1-27
mAbs	δ2-17	15D	δ 2-32	δ 2-14
	B6			δ 2-22
				δ 2-30
				δ 2-31
				δ 2-36
				δ 2-37

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* * *

While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is

CLAIMS

WHAT IS CLAIMED IS:

1. An *ex vivo* method for producing an enriched δ1 γδ T-cell population from an isolated mixed cell population without prior selection of γδ T-cells and/or depletion of non-γδ T-cells, and under culture conditions that do not comprise IL-4 and wherein said cell population has not been exposed to exogenous IL-4 prior to expansion, said method comprising directly contacting the mixed cell population with at least one antibody that selectively expands δ1 T-cells without significant expansion of δ2 T-cells by binding to an epitope specific to a δ1 variable region of a δ1 TCR to provide clinically-relevant levels of an enriched δ1 γδ T cell population, wherein the at least one antibody comprises a heavy chain variable region/light chain variable region (HCVR/LCVR) sequence pair selected from the group consisting of SEQ ID NOs: 153/180, 154/181, 155/182, 156/183, 157/184, 158/185, 159/186, 160/187, 161/188, 162/189, 163/190, 164/191, 165/192, 166/193, 167/194, 168/195, 169/196, 170/197, 171/198, 172/199, 173/200, 174/201, 175/202, 176/203, 177/204, 178/205, and 179/206.

2. The method of claim 1, wherein the percentage of δ1 T cells in the enriched γδ T cell population is greater than 60% δ1 T-cells of total viable cells.

3. The method of any one of the preceding claims, wherein said method does not comprise expanding the enriched δ1 γδ T-cell population by an antigen presenting cell or an aminophosphate.

4. The method of claim 1, wherein said at least one antibody that selectively expands δ1 T-cells is immobilized on a surface.

5. The method of claim 1, wherein said isolated mixed cell population is selected from a peripheral blood sample, a cord blood sample or a tumor.

6. The method of claim 1, wherein said enriched δ1 γδ T-cell population comprise polyclonal TCR diversity.

7. The method of claim 1, wherein the enriched δ1 γδ T-cell population is further formulated for administration to a subject.

8. The method of claim 1, wherein the enriched δ1 γδ T-cell population includes a therapeutically effective amount of γδ T-cells.

9. The method of claim 1, wherein said δ1 γδ T-cell population is further engineered to stably express one or more tumor recognition moieties.

10. The method of claim 1, further comprising:

directly contacting at least a portion of the enriched δ1 γδ T-cell population with antigen presenting cells (APCs) in a second γδ T-cell expansion, thereby producing a second enriched γδ T-cell population,

wherein the second enriched γδ T-cell population comprises a clinically relevant number of γδ T-cells.

11. The method of claim 1, further comprising:

directly contacting at least a portion of the enriched δ1 γδ T-cell population with one or more agents that (a) expand γδ T-cells, and/or (b) deplete αβ T-cells.

12. The method of claim 1, wherein an antibody that selectively expands δ1 T-cells is an antibody that specifically recognizes the δ1 chain variable region and i) the CDR3 loop created by the restricted juxtaposition of V(D)J1 and V(D)J2 but not V(D)J3 segments, or iii) the CDR3 loop created by the juxtaposition of V(D)J1, V(D)J2 and V(D)J3 segments.

13. The method of claim 1, wherein the antibody binds to an epitope comprising residues Arg71, Asp72 and Lys120 of the δ1 variable region.

14. The method of claim 1, wherein the antibody has reduced binding to a mutant 61 TCR polypeptide comprising a mutation at K120 at the delta J1 and delta J2.

15. The method of claim 1, wherein the epitope is selected from a δ1 TCR Bin 1 δ1 epitope, Bin 1b δ1 epitope, Bin 2 δ1 epitope, Bin 2b δ1 epitope, Bin 2c δ1 epitope, Bin 3 δ1 epitope, Bin 4 δ1 epitope, Bin 5 δ1 epitope, Bin 6 δ1 epitope, Bin 7 δ1 epitope, Bin 8 δ1 epitope, or Bin 9 δ1 epitope of a human δ1 TCR.