

THERAPEUTIC USES OF ANTI-TCR DELTA VARIABLE 1 ANTIBODIES

FIELD OF THE INVENTION

The invention relates therapeutic uses of antibodies and fragments thereof directed to the T
5 cell receptor of gamma delta T cells.

BACKGROUND OF THE INVENTION

The growing interest in T cell immunotherapy for cancer has focused on the evident capacity
of subsets of CD8+ and CD4+ alpha beta ($\alpha\beta$) T cells to recognize cancer cells and to mediate
10 host-protective functional potentials, particularly when de-repressed by clinically mediated
antagonism of inhibitory pathways exerted by PD-1, CTLA-4, and other receptors. However,
 $\alpha\beta$ T cells are MHC-restricted which can lead to graft versus host disease.

Gamma delta T cells ($\gamma\delta$ T cells) represent a subset of T cells that express on their surface a
15 distinct, defining $\gamma\delta$ T-cell receptor (TCR). This TCR is made up of one gamma (γ) and one
delta (δ) chain, each of which undergoes chain rearrangement but have a limited number of V
genes as compared to $\alpha\beta$ T cells. The main TRGV gene segments encoding $V\gamma$ are TRGV2,
TRGV3, TRGV4, TRGV5, TRGV8, TRGV9 and TRGV11 and non-functional genes TRGV10,
TRGV11, TRGVA and TRGVB. The most frequent TRDV gene segments encode $V\delta$ 1, $V\delta$ 2,
20 and $V\delta$ 3, plus several V segments that have both $V\delta$ and $V\alpha$ designation (Adams *et al.*,
296:30-40 (2015) *Cell Immunol.*). Human $\gamma\delta$ T cells can be broadly classified based on their
TCR chains, as certain γ and δ types are found on cells more prevalently, though not
exclusively, in one or more tissue types. For example, most blood-resident $\gamma\delta$ T cells express
a $V\delta$ 2 TCR, commonly $V\gamma$ 9 $V\delta$ 2, whereas this is less common among tissue-resident $\gamma\delta$ T cells
25 such as those in the skin, which more frequently use the $V\delta$ 1 TCR paired with gamma chains,
for example often paired with $V\gamma$ 4 in the gut.

To exploit $\gamma\delta$ T cells for immunotherapy requires either a means to expand the cells *in situ* or
to harvest them and expand them *ex vivo* prior to re-infusion. The latter approach has
30 previously been described using the addition of exogenous cytokines, for example see
WO2017/072367 and WO2018/212808. Methods for expanding a patients' own $\gamma\delta$ T cells has
been described using pharmacologically modified forms of hydroxy-methyl but-2-enyl
pyrophosphate (HMBPP) or clinically-approved aminobisphosphonates. By these
approaches, over 250 cancer patients have been treated, seemingly safely, but with only rare
35 incidences of complete remission. However, there is still a need for activating agents that have
the proven capacity to expand large numbers of $\gamma\delta$ T cells.

Further, a binding or activating agent capable of preferentially targeting or binding or recognizing or specifically modulating or increasing the numbers of V δ 1+ cells *in-situ* may be highly desirable as a medicament.

- 5 However, whilst medicaments exist that do potentially modulate V δ 2+ cells inclusive of the aminobisphosphonates such as Zometa® (zoledronic acid), said medicines are primarily designed to slow bone reabsorption. And regardless of said V δ 2+ modulation, there is a need to develop medicines specifically designed to bind, target, modulate, activate, or increase the numbers of V δ 1+ cells.

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- Further, and given the predominate tissue-resident nature of V δ 1+ cells, an ideal medicament capable of modulating V δ 1+ would also exhibit fewer 'off-target' undesirable effects and rapid renal clearance. Typically, said undesirable effects can manifest when employing small-molecule chemicals. For example, the aforementioned aminobisphosphonates shown capable of modulating the separate class of V δ 2+ cells (as a secondary effect versus primary modulating effect on bone) are associated with renal toxicity which manifests as deterioration of renal function and potential renal failure (e.g. Markowitz et al. (2003) *Kidney Int.* 64(1):281-289). Additional undesirable effects as listed by the European Medicine Agency for Zometa include anaemia, hypersensitivity reactions, hypertension, arterial fibrillation, myalgia, general pain, malaise, blood urea increase, vomiting, joint swelling, chest pain, etc.

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- Hence there is a need for improved medicaments specifically designed to target V δ 1+ cells and for the treatment of infections, autoimmune conditions, and cancer. Specifically, there is a need for medicaments that can be administered to ameliorate signs and symptoms of disease by specifically binding V δ 1+ cells, targeting V δ 1+ cells, specifically activating V δ 1+ cells, specifically enhancing proliferation and/or cytotoxicity activity V δ 1+ cells, or specifically blocking activation of V δ 1+ cells.

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SUMMARY OF THE INVENTION

- 30 According to a first aspect, there is provided an anti-v δ 1 antibody or fragment thereof for use in a method of treating cancer, an infectious disease or an inflammatory disease. It will be understood that the methods and compositions for use described herein relate to the administration of the anti-v δ 1 antibody or fragment thereof directly to the subject to be treated.

- 35 According to a further aspect of the invention, there is provided an isolated multi-specific antibody or fragment thereof that binds to at least two target antigens, wherein the first of the at least two target antigens is V δ 1, and which comprises one or more of:

a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25;

a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3); and/or

5 a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.

According to a further aspect of the invention, there is provided a human, isolated anti-TCR delta variable 1, multi-specific antibody or fragment thereof, that binds to at least two target
10 antigens, wherein the first of the at least two target antigens is V δ 1, and wherein the multi-specific antibody or fragment thereof which binds to an epitope of V δ 1 comprising one or more amino acid residues within amino acids 1-90 of SEQ ID NO: 1.

According to a further aspect of the invention, there is provided an isolated multi-specific
15 antibody or fragment thereof as defined herein for use as a medicament.

According to a further aspect of the invention, there is provided an isolated multi-specific antibody or fragment thereof as defined herein for use in the treatment of cancer, an infectious disease or an inflammatory disease.

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BRIEF DESCRIPTION OF THE FIGURES

Figure 1: ELISA Detection of Directly Coated Antigen with Anti-V δ 1Ab (REA173, Miltenyi Biotec). Detection was seen only with those antigens which contain the V δ 1 domain. Leucine zipper (LZ) format seems more potent than Fc format which is consistent with cell-
25 based flow competition assay (data not shown).

Figure 2: Polyclonal phage DELFIA data for DV1 selections. A) Heterodimer selections: heterodimeric LZ TCR format in round 1 and 2, with deselections on heterodimeric LZ TCR in both rounds. B) Homodimer selections: round 1 performed using homodimeric Fc
30 fusion TCR with deselection on human IgG1 Fc followed by round 2 on heterodimeric LZ TCR with deselection on heterodimeric LZ TCR. Each graph contains two bars for each target to represent selections from different libraries.

Figure 3: IgG capture: left) Sensorgrams of interaction of anti-L1 IgG with L1, right) steady state fits, if available. All experiments were performed at room temperature on MASS-
35 2 instrument. Steady state fitting according to Langmuir 1:1 binding.

Figure 4: Results of TCR Downregulation Assay for clones 1245_P01_E07, 1252_P01_C08, 1245_P02_G04, 1245_P01_B07 and 1251_P02_C05 (A) or clones 1139_P01_E04, 1245_P02_F07, 1245_P01_G06 1245_P01_G09, 1138_P01_B09, 1251_P02_G10 and 1252_P01_C08 (B).

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Figure 5: Results of T cell degranulation Assay for clones 1245_P01_E07, 1252_P01_C08, 1245_P02_G04, 1245_P01_B07 and 1251_P02_C05 (A) or clones 1139_P01_E04, 1245_P02_F07, 1245_P01_G06, 1245_P01_G09, 1138_P01_B09, and 1251_P02_G10 (B).

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Figure 6: Results of Killing Assay (THP-1 flow-based assay) for clones 1245_P01_E07, 1252_P01_C08, 1245_P02_G04, 1245_P01_B07 and 1251_P02_C05 (A) or clones 1139_P01_E04, 1245_P02_F07, 1245_P01_G06, 1245_P01_G09, 1138_P01_B09 and 1251_P02_G10 (B).

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Figure 7: Epitope mapping data for 1245_P01_E07. Graphical representation of epitope binding site of 1245_P01_E07 on SEQ ID NO: 1.

Figure 8: Epitope mapping data for 1252_P01_C08. Graphical representation of epitope binding site of 1252_P01_C08 on SEQ ID NO: 1.

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Figure 9: Epitope mapping data for 1245_P02_G04. Graphical representation of epitope binding site of 1245_P02_G04 on SEQ ID NO: 1.

Figure 10: Epitope mapping data for 1251_P02_C05. Graphical representation of epitope binding site of 1251_P02_C05 on SEQ ID NO: 1.

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Figure 11: Epitope mapping data for 1141_P01_E01. Graphical representation of epitope binding site of 1141_P01_E01 on SEQ ID NO: 1.

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Figure 12: Total cell counts during Experiment 1 of Example 10. Samples were cultured with varying concentration of anti-V δ 1 antibodies described herein and compared to samples cultured with comparator antibodies or controls. Graphs show total cell counts at (A) day 7, (B) day 14 and (C) day 18.

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Figure 13: Analysis of V δ 1 T cells during Experiment 1 of Example 10. Graphs show (A) percentage of V δ 1 T cells, (B) V δ 1 T cell count and (C) V δ 1 fold change in the samples at day 18.

5 **Figure 14: Total cell counts during Experiment 2 of Example 10.** Samples were cultured with varying concentration of anti-V δ 1 antibodies described herein and compared to samples cultured with comparator antibodies or controls. Graphs show total cell counts at (A) day 7, (B) day 11, (C) day 14 and (D) day 17.

10 **Figure 15: Analysis of V δ 1 T cells during Experiment 2 of Example 10.** Graphs show (A) percentage of V δ 1 T cells, (B) V δ 1 T cell count and (C) V δ 1 fold change in the samples at day 17.

15 **Figure 16: Cell composition analysis.** The cell types present in the samples (including non-V δ 1 cells) were measured on day 17 of Experiment 2. Cells were harvested and analysed by flow cytometry for surface expression of V δ 1, V δ 2 and $\alpha\beta$ TCR. The percentage values are also provided in **Table 6**.

20 **Figure 17: SYTOX-flow killing assay results.** Cell functionality was tested using the SYTOX-flow killing assay and results are presented for (A) Experiment 1 at day 14 using cells in a 10:1 Effector-to-Target (E:T) ratio, and (B) Experiment 2 at day 17 (post freeze-thaw) using cells at a 1:1 and 10:1 E:T ratio.

25 **Figure 18: Total cell count post freeze-thaw.** Graph shows the total cell counts after 7 days of culturing cells post freeze-thaw for cultures contacted with B07, C08, E07, G04 or OKT-3 antibodies prior to freezing.

30 **Figure 19: Monitoring cell expansion.** Total cell counts were monitored until day 42 for cells cultured post freeze-thaw.

Figure 20: Binding equivalence studies on modified anti-V δ 1 antibodies.

Figure 21: Anti-V δ 1 antibody binding equivalence studies on human germline V δ 1 antigen and a polymorphic variant thereof.

Figure 22: Anti-V δ 1 antibody conferred increases in V δ 1+ cell cytokine secretion levels.

Tissue-derived $\gamma\delta$ T cells incubated with the antibodies as indicated. **A)** The levels of TNF-alpha observed **B)** The levels of IFN-gamma observed.

- 5 **Figure 23: Anti-V δ 1 antibody conferred increases in V δ 1+ cell Granzyme B levels/activity** Cancer cells co-cultured with tissue-derived $\gamma\delta$ T cells for one hour at a set 1:20 T:E ratio and with the antibodies as indicated. Results highlight the quantities of Granzyme B detected in the cancer cells at the end of co-culture.

- 10 **Figure 24: Anti-V δ 1 antibody conferred modulation and proliferation of immune cells in human tissue.** Human skin punch-biopsies (from five different donors) incubated for 21-days in culture with the antibodies as indicated. **A)** The number of viable pan- $\gamma\delta$ + cells. **B)** The number of viable V δ 1+ cells. **C)** The percentage of viable, double-positive V δ 1+ CD25+ cells.

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- Figure 25: Anti-V δ 1 antibody conferred modulation and proliferation of tumour-infiltrating-lymphocyte (TILs) in human tumours.** Studies on renal cell carcinoma (RCC) +/- antibodies **A)** Fold-increase in TIL V δ 1+ cells. **B)** Total numbers of TIL V δ 1+ cells. **C)** Example gating strategy **D)** Comparative cell-surface phenotypic profile of TIL V δ 1+ cells. **E)** Analysis of the TIL V δ 1-negative gated fraction.

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- Figure 26: Anti-V δ 1 antibody conferred enhancement of V δ 1+ mediated cytotoxicity, and diseased-cell-specific cytotoxicity.** Cytotoxicity/potency-assays in model systems comprising a triculture of V δ 1+ effector cells, THP-1 monocytic cancer cells, and non-diseased, healthy primary monocytes. **A)** Quantification of THP-1 and monocyte cell numbers in triple co-culture with $\gamma\delta$ T-cells in the presence of anti-V δ 1 mAbs or controls. **B)** A bar chart representation highlighting the window between diseased-cell specific killing and non-diseased healthy: Left-hand bar chart; fold-increase in killing of diseased-cells (THP-1) versus killing of non-diseased cells (primary human monocytes); Right-hand bar chart; same data but represented as percent-enhanced killing versus controls **C)** Tabulated results summarizing the percent improvement in potency of V δ 1+ effector cell killing of THP-1 target cells +/- mAbs. **D)** Tabulated results of EC50 values as calculated from Figure (A) represented as $\gamma\delta$ T-cell numbers required to confer 50% THP-1 cell killing.

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- 35 **Figure 27: Multi-specific antibody conferred enhancement of V δ 1+ effector cell mediated cytotoxicity.** The targeting of a tissue-centric disease associated antigen: **(A-D)** Example co-culture of V δ 1+ effector cells with A-431 cancer cells +/- multi-specific antibodies

comprising anti-V δ 1 x anti-TAA (EGFr) bispecific binding moieties wherein the anti-V δ 1 VL+VH binding domain (to the first target) is combined with the CH1-CH2-CH3 domain of an anti-EGFr binding moiety (to the second target). **(E-H)** Example co-culture of V δ 1+ effector cells with A-431 cancer cells +/- multi-specific antibodies comprising anti-V δ 1 x anti-TAA (EGFr) bispecific binding moieties wherein the anti-V δ 1 binding domain (to the first target) comprises a full-length antibody (VH-CH1-CH2-CH3/VL-CL) then combined with an anti-EGFr cetuximab-derived scFv binding moiety (to the second target). **(I-J)** Alternative approach to representing the data: Percentage improvement conferred by multi-specific antibodies upon V δ 1+ effector cell cytotoxicity towards EGFR+ cells relative to component parts.

Figure 28: Multi-specific antibody conferred enhancement of V δ 1+ mediated cytotoxicity and diseased-cell-specific cytotoxicity. The targeting of a hemopoietic disease associated antigen **(A)** E:T ratios required to induce 50% Raji cell killing **(B)** Percentage improvement with addition of a V δ 1-CD19 multi-specific antibodies

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below.

Gamma delta ($\gamma\delta$) T cells represent a small subset of T cells that express on their surface a distinct, defining T Cell Receptor (TCR). This TCR is made up of one gamma (γ) and one delta (δ) chain. Each chain contains a variable (V) region, a constant (C) region, a transmembrane region and a cytoplasmic tail. The V region contains an antigen binding site. There are two major sub-types of human $\gamma\delta$ T cells: one that is dominant in the peripheral blood and one that is dominant in non-haematopoietic tissues. The two sub-types may be defined by the type of δ and/or γ present on the cells. For example, $\gamma\delta$ T cells that are dominant in peripheral blood primarily express the delta variable 2 chain (V δ 2). $\gamma\delta$ T cells that are dominant in non-haematopoietic tissues (*i.e.* are tissue-resident) primarily express the delta variable 1 chain. References to "V δ 1 T cells" refer to $\gamma\delta$ T cells with a V δ 1 chain, *i.e.* V δ 1+ cells.

References to "delta variable 1" may also referred to as V δ 1 or Vd1, while a nucleotide encoding a TCR chain containing this region may be referred to as "TRDV1". Antibodies or fragments thereof which interact with the V δ 1 chain of a $\gamma\delta$ TCR, are all effectively antibodies

or fragments thereof which bind to V δ 1 and may be referred to as “anti-TCR delta variable 1 antibodies or fragments thereof” or “anti-V δ 1 antibodies or fragments thereof”.

Additional references are made herein to other delta chains such as the “delta variable 2” chain. These can be referred to in a similar manner. For example, delta variable 2 chains can be referred to as V δ 2, while a nucleotide encoding a TCR chain containing this region may be referred to as “TRDV2”. In preferred embodiments antibodies or fragments thereof which interact with the V δ 1 chain of a $\gamma\delta$ TCR, do not interact with other delta chains such as V δ 2

References to ‘gamma variable chains’ are also made herein. These may be referred to as γ -chains or V γ , while a nucleotide encoding a TCR chain containing this region may be referred to as TRGV. For example, TRGV4 refers to V γ 4 chain. In preferred embodiments, antibodies or fragments thereof which interact with the V δ 1 chain of a $\gamma\delta$ TCR, do not interact with gamma chains such as V γ 4.

The term “antibody” includes any antibody protein construct comprising at least one antibody variable domain comprising at least one antigen binding site (ABS). Antibodies include, but are not limited to, immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). The overall structure of Immunoglobulin G (IgG) antibodies assembled from two identical heavy (H)-chain and two identical light (L)-chain polypeptides is well established and highly conserved in mammals (Padlan (1994) *Mol. Immunol.* 31:169-217).

A conventional antibody or immunoglobulin (Ig) is a protein comprising four polypeptide chains: two heavy (H) chains and two light (L) chains. Each chain is divided into a constant region and a variable domain. The heavy (H) chain variable domains are abbreviated herein as V_H, and the light (L) chain variable domains are abbreviated herein as V_L. These domains, domains related thereto and domains derived therefrom, may be referred to herein as immunoglobulin chain variable domains. The V_H and V_L domains (also referred to as V_H and V_L regions) can be further subdivided into regions, termed “complementarity determining regions” (“CDRs”), interspersed with regions that are more conserved, termed “framework regions” (“FRs”). The framework and complementarity determining regions have been precisely defined (Kabat *et al.* Sequences of Proteins of Immunological Interest, *Fifth Edition* U.S. Department of Health and Human Services, (1991) NIH Publication Number 91-3242). There are also alternative numbering conventions for CDR sequences, for example those set out in Chothia *et al.* (1989) *Nature* 342: 877-883. In a conventional antibody, each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The conventional antibody

tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains is formed with the heavy and the light immunoglobulin chains inter-connected by e.g. disulphide bonds, and the heavy chains similarly connected. The heavy chain constant region includes three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable domain of the heavy chains and the variable domain of the light chains are binding domains that interact with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g. effector cells) and the first component (C1q) of the classical complement system.

A fragment of the antibody (which may also referred to as "antibody fragment", "immunoglobulin fragment", "antigen-binding fragment" or "antigen-binding polypeptide") as used herein refers to a portion of an antibody (or constructs that contain said portion) that specifically binds to the target, the delta variable 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (e.g. a molecule in which one or more immunoglobulin chains is not full length, but which specifically binds to the target). Examples of binding fragments encompassed within the term antibody fragment include:

- (i) a Fab fragment (a monovalent fragment consisting of the VL, VH, CL and CH1 domains);
- (ii) a F(ab')₂ fragment (a bivalent fragment consisting of two Fab fragments linked by a disulphide bridge at the hinge region);
- (iii) a Fd fragment (consisting of the VH and CH1 domains);
- (iv) a Fv fragment (consisting of the VL and VH domains of a single arm of an antibody);
- (v) a single chain variable fragment, scFv (consisting of VL and VH domains joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules);
- (vi) a VH (an immunoglobulin chain variable domain consisting of a VH domain);
- (vii) a VL (an immunoglobulin chain variable domain consisting of a VL domain);
- (viii) a domain antibody (dAb, consisting of either the VH or VL domain);
- (ix) a minibody (consisting of a pair of scFv fragments which are linked via CH3 domains);
- and
- (x) a diabody (consisting of a noncovalent dimer of scFv fragments that consist of a VH domain from one antibody connected by a small peptide linker a VL domain from another antibody).

"Human antibody" refers to antibodies having variable and constant regions derived from human germline immunoglobulin sequences. Human subjects administered with said human antibodies do not generate cross-species antibody responses (for example termed HAMA

- responses - human-anti-mouse antibody) to the primary amino acids contained within said antibodies. Said human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g. mutations introduced by random or site-specific mutagenesis or by somatic mutation), for example in the CDRs and in particular CDR3.
- 5 However, the term is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human
- 10 antibody library, antibodies isolated from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences, may also be referred to as "recombinant human antibodies".
- 15 Substituting at least one amino acid residue in the framework region of a non-human immunoglobulin variable domain with the corresponding residue from a human variable domain is referred to as "humanisation". Humanisation of a variable domain may reduce immunogenicity in humans.
- 20 "Specificity" refers to the number of different types of antigens or antigenic determinants to which a particular antibody or fragment thereof can bind. The specificity of an antibody is the ability of the antibody to recognise a particular antigen as a unique molecular entity and distinguish it from another. An antibody that "specifically binds" to an antigen or an epitope is a term well understood in the art. A molecule is said to exhibit "specific binding" if it reacts
- 25 more frequently, more rapidly, with greater duration and/or with greater affinity with a particular target antigen or epitope, than it does with alternative targets. An antibody "specifically binds" to a target antigen or epitope if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances.
- 30 "Affinity", represented by the equilibrium constant for the dissociation of an antigen with an antigen-binding polypeptide (KD), is a measure of the binding strength between an antigenic determinant and an antigen-binding site on the antibody (or fragment thereof): the lesser the value of the KD, the stronger the binding strength between an antigenic determinant and the antigen-binding polypeptide. Alternatively, the affinity can also be expressed as the affinity
- 35 constant (KA), which is 1/KD. Affinity can be determined by known methods, depending on the specific antigen of interest.

Any KD value less than 10^{-6} is considered to indicate binding. Specific binding of an antibody, or fragment thereof, to an antigen or antigenic determinant can be determined in any suitable known manner, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition
5 assays, equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (e.g. using a fluorescence assay) and the different variants thereof known in the art.

“Avidity” is the measure of the strength of binding between an antibody, or fragment thereof,
10 and the pertinent antigen. Avidity is related to both the affinity between an antigenic determinant and its antigen binding site on the antibody and the number of pertinent binding sites present on the antibody.

“Human tissue Vδ1+ cells,” and “haemopoietic and blood Vδ1+ cells” and “tumour infiltrating
15 lymphocyte (TIL) Vδ1+ cells,” are defined as Vδ1+ cells contained in or derived from either human tissue or the haemopoietic blood system or human tumours respectively. All said cell types can be identified by their (i) location or from where they are derived and (ii) their expression of the Vδ1+ TCR.

20 “Modulating antibodies” are antibodies that confer a measurable change including, but not limited to, a measurable change in cell cycle, and/or in cell number, and/or cell viability, and/or in one or more cell surface markers, and/or in the secretion of one or more secretory molecules (e.g., cytokines, chemokines, leukotrienes, etc.), and/or a function (such as cytotoxicity
25 towards a target cell or diseased cell), upon contacting or binding to a cell expressing the target to which the antibody binds. A method of “modulating” a cell, or population thereof, refers to a method wherein in at least one measurable change in said cell or cells, or secretion therefrom, is triggered to generate one or more “modulated cells”.

An “immune response” is a measurable change in at least one cell, or one cell-type, or one
30 endocrine pathway, or one exocrine pathway, of the immune system (including but not limited to a cell-mediated response, a humoral response, a cytokine response, a chemokine response) upon addition of a modulating antibody.

An “immune cell” is defined as a cell of the immune system including, but not limited to, CD34+
35 cells, B-Cells, CD45+ (lymphocyte common antigen) cells, Alpha-Beta T-cells, Cytotoxic T-cells, Helper T-cells, Plasma Cells, Neutrophils, Monocytes, Macrophages, Red Blood Cells, Platelets, Dendritic Cells, Phagocytes, Granulocytes, Innate lymphoid cells, Natural Killer (NK)

cells and Gamma Delta T-cells. Typically, immune cells are classified with the aid of combinatorial cell surface molecule analysis (e.g., via flow cytometry) to identify or group or cluster to differentiate immune cells into sub-populations. These can be then still further sub-divided with additional analysis. For example, CD45+ lymphocytes can further sub-divided into
5 v δ positive populations and v δ negative populations.

“Model systems” are biological models or biological representations designed to aid in the understanding of how a medicine such as an antibody or fragment thereof may function as a medicament in the amelioration of a sign or symptom of disease. Such models typically include
10 the use of *in vitro*, *ex vivo*, and *in vivo* diseased cells, non-diseased cells, healthy cells, effector cells, and tissues etc., and in which the performance of said medicaments are studied and compared.

“Diseased cells” exhibit a phenotype associated with the progression of a disease such as a cancer, an infection such as a viral infection, or an inflammatory condition or inflammatory disease. For example, a diseased cell may be a tumour cell, an autoimmune tissue cell or a virally infected cell. Accordingly said diseased cells may be defined as tumorous, or virally infected, or inflammatory.

“Healthy cells” refers to normal cells that are not diseased. They may also be referred to as “normal” or “non-diseased” cells. Non-diseased cells include non-cancerous, or non-infected, or non-inflammatory cells. Said cells are often employed alongside relevant diseased cells to determine the diseased cell specificity conferred by a medicament and/or better understand the therapeutic index of a medicament.

“Diseased-cell-specificity” is a measure of how effective an effector cell or population thereof, (such as, for example, a population of V δ 1+ cells) is at distinguishing and killing diseased cells, such as cancer cells, whilst sparing non-diseased or healthy cells. This potential can be measured in model systems and may involve comparing the propensity of an effector cell, or
25 a population of effector cells, to selectively kill or lyse diseased cells versus the potential of said effector cell/s to kill or lyse non-diseased or healthy cells. Said diseased-cell-specificity can inform the potential therapeutic index of a medicament.

“Enhanced diseased-cell specificity” describes a phenotype of an effector cell such as, for example, a V δ 1+ cell, or population thereof, which has been modulated to further increase its capacity to specifically kill diseased cells. This enhancement can be measured in a variety of
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ways inclusive of fold-change, or percentage increase, in diseased-cell killing specificity or selectivity.

Suitably, the antibody or fragment thereof (*i.e.* polypeptide) of the invention is isolated. An “isolated” polypeptide is one that is removed from its original environment. The term “isolated” may be used to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.* an isolated antibody that specifically binds V δ 1, or a fragment thereof, is substantially free of antibodies that specifically bind antigens other than V δ 1). The term “isolated” may also be used to refer to preparations where the isolated antibody is sufficiently pure to be administered therapeutically when formulated as an active ingredient of a pharmaceutical composition, or at least 70-80% (w/w) pure, more preferably, at least 80-90% (w/w) pure, even more preferably, 90-95% pure; and, most preferably, at least 95%, 96%, 97%, 98%, 99%, or 100% (w/w) pure.

Suitably, the polynucleotides used in the present invention are isolated. An “isolated” polynucleotide is one that is removed from its original environment. For example, a naturally-occurring polynucleotide is isolated if it is separated from some or all of the coexisting materials in the natural system. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of its natural environment or if it is comprised within cDNA.

The antibody or fragment thereof may be a “functionally active variant” which also includes naturally occurring allelic variants, as well as mutants or any other non-naturally occurring variants. As is known in the art, an allelic variant is an alternate form of a (poly)peptide that is characterized as having a substitution, deletion, or addition of one or more amino acids that does essentially not alter the biological function of the polypeptide. By way of non-limiting example, said functionally active variants may still function when the frameworks containing the CDRs are modified, when the CDRs themselves are modified, when said CDRs are grafted to alternate frameworks, or when N- or C-terminal extensions are incorporated. Further, CDR containing binding domains may be paired with differing partner chains such as those shared with another antibody. Upon sharing with so called ‘common’ light or ‘common’ heavy chains, said binding domains may still function. Further, said binding domains may function when multimerized. Further, ‘antibodies or fragments thereof’ may also comprise functional variants wherein the VH or VL or constant domains have been modified away or towards a different canonical sequence (for example as listed at IMGT.org) and which still function.

For the purposes of comparing two closely-related polypeptide sequences, the “% sequence identity” between a first polypeptide sequence and a second polypeptide sequence may be calculated using NCBI BLAST v2.0, using standard settings for polypeptide sequences (BLASTP). For the purposes of comparing two closely-related polynucleotide sequences, the “% sequence identity” between a first nucleotide sequence and a second nucleotide sequence may be calculated using NCBI BLAST v2.0, using standard settings for nucleotide sequences (BLASTN).

Polypeptide or polynucleotide sequences are said to be the same as or “identical” to other polypeptide or polynucleotide sequences, if they share 100% sequence identity over their entire length. Residues in sequences are numbered from left to right, *i.e.* from N- to C-terminus for polypeptides; from 5' to 3' terminus for polynucleotides.

A “difference” between sequences refers to an insertion, deletion or substitution of a single amino acid residue in a position of the second sequence, compared to the first sequence. Two polypeptide sequences can contain one, two or more such amino acid differences. Insertions, deletions or substitutions in a second sequence which is otherwise identical (100% sequence identity) to a first sequence result in reduced % sequence identity. For example, if the identical sequences are 9 amino acid residues long, one substitution in the second sequence results in a sequence identity of 88.9%. If first and second polypeptide sequences are 9 amino acid residues long and share 6 identical residues, the first and second polypeptide sequences share greater than 66% identity (the first and second polypeptide sequences share 66.7% identity).

Alternatively, for the purposes of comparing a first, reference polypeptide sequence to a second, comparison polypeptide sequence, the number of additions, substitutions and/or deletions made to the first sequence to produce the second sequence may be ascertained. An “addition” is the addition of one amino acid residue into the sequence of the first polypeptide (including addition at either terminus of the first polypeptide). A “substitution” is the substitution of one amino acid residue in the sequence of the first polypeptide with one different amino acid residue. Said substitution may be conservative or non-conservative. A “deletion” is the deletion of one amino acid residue from the sequence of the first polypeptide (including deletion at either terminus of the first polypeptide).

A “conservative” amino acid substitution is an amino acid substitution in which an amino acid residue is replaced with another amino acid residue of similar chemical structure and which is expected to have little influence on the function, activity or other biological properties of the

polypeptide. Such conservative substitutions suitably are substitutions in which one amino acid within the following groups is substituted by another amino acid residue from within the same group:

Group	Amino acid residue
Non-polar aliphatic	Glycine
	Alanine
	Valine
	Methionine
	Leucine
	Isoleucine
Aromatic	Phenylalanine
	Tyrosine
	Tryptophan
Polar uncharged	Serine
	Threonine
	Cysteine
	Proline
	Asparagine
	Glutamine
Negatively charged	Aspartate
	Glutamate
Positively charged	Lysine
	Arginine
	Histidine

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Suitably, a hydrophobic amino acid residue is a non-polar amino acid. More suitably, a hydrophobic amino acid residue is selected from V, I, L, M, F, W or C.

As used herein, numbering of polypeptide sequences and definitions of CDRs and FRs are as defined according to the Kabat system (Kabat *et al.*, 1991, herein incorporated by reference in its entirety). A “corresponding” amino acid residue between a first and second polypeptide sequence is an amino acid residue in a first sequence which shares the same position according to the Kabat system with an amino acid residue in a second sequence, whilst the amino acid residue in the second sequence may differ in identity from the first. Suitably corresponding residues will share the same number (and letter) if the framework and CDRs are the same length according to Kabat definition. Alignment can be achieved manually or by using, for example, a known computer algorithm for sequence alignment such as NCBI BLAST v2.0 (BLASTP or BLASTN) using standard settings.

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References herein to an “epitope” refer to the portion of the target which is specifically bound by the antibody or fragment thereof. Epitopes may also be referred to as “antigenic determinants”. An antibody binds “essentially the same epitope” as another antibody when they both recognize identical or sterically overlapping epitopes. Commonly used methods to determine whether two antibodies bind to identical or overlapping epitopes are competition assays, which can be configured in a number of different formats (e.g. well plates using radioactive or enzyme labels, or flow cytometry on antigen-expressing cells) using either labelled antigen or labelled antibody.

Epitopes found on protein targets may be defined as “linear epitopes” or “conformational epitopes”. Linear epitopes are formed by a continuous sequence of amino acids in a protein antigen. Conformational epitopes are formed of amino acids that are discontinuous in the protein sequence, but which are brought together upon folding of the protein into its three-dimensional structure.

The term “vector”, as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid”, which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian and yeast vectors). Other vectors (e.g. non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply, “expression vectors”). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions, and also bacteriophage and phagemid systems. The term “recombinant host cell” (or simply “host cell”), as used herein, is intended to refer to a cell into which a recombinant expression vector has been introduced. Such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell, for example, when said progeny are employed to make a cell line or cell bank which is then optionally stored, provided, sold, transferred, or employed to manufacture an antibody or fragment thereof as described herein.

References to “subject”, “patient” or “individual” refer to a subject, in particular a mammalian subject, to be treated. Mammalian subjects include humans, non-human primates, farm animals (such as cows), sports animals, or pet animals, such as dogs, cats, guinea pigs, rabbits, rats or mice. In some embodiment, the subject is a human. In alternative
5 embodiments, the subject is a non-human mammal, such as a mouse.

The term “sufficient amount” means an amount sufficient to produce a desired effect. The term “therapeutically effective amount” is an amount that is effective to ameliorate a symptom of a
10 disease or disorder. A therapeutically effective amount can be a “prophylactically effective amount” as prophylaxis can be considered therapy.

A disease or disorder is “ameliorated” if the severity of a sign or symptom of the disease or disorder, the frequency with which such a sign or symptom is experienced by a subject, or
15 both, is reduced.

As used herein, “treating a disease or disorder” means reducing the frequency and/or severity of at least one sign or symptom of the disease or disorder experienced by a subject.

20 “Cancer,” as used herein, refers to the abnormal growth or division of cells. Generally, the growth and/or life span of a cancer cell exceeds, and is not coordinated with, that of the normal cells and tissues around it. Cancers may be benign, pre-malignant or malignant. Cancer occurs in a variety of cells and tissues, including the oral cavity (e.g., mouth, tongue, pharynx, etc.), digestive system (e.g., esophagus, stomach, small intestine, colon, rectum, liver, bile
25 duct, gall bladder, pancreas, etc.), respiratory system (e.g., larynx, lung, bronchus, etc.), bones, joints, skin (e.g., basal cell, squamous cell, meningioma, etc.), breast, genital system, (e.g., uterus, ovary, prostate, testis, etc.), urinary system (e.g., bladder, kidney, ureter, etc.), eye, nervous system (e.g., brain, etc.), endocrine system (e.g., thyroid, etc.), and hematopoietic system (e.g., lymphoma, myeloma, leukemia, acute lymphocytic leukemia,
30 chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, etc.).

As used herein, the term “about” when used herein includes up to and including 10% greater and up to and including 10% lower than the value specified, suitably up to and including 5% greater and up to and including 5% lower than the value specified, especially the value
35 specified. The term “between”, includes the values of the specified boundaries.

Antibodies or fragments thereof

Provided herein are antibodies or fragments thereof capable of specifically binding to the delta variable 1 chain (V δ 1) of a $\gamma\delta$ T Cell Receptor (TCR). The invention relates to the use of said antibodies as medicaments for administration to a subject to be treated.

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In one embodiment, the antibody or fragment thereof is an scFv, Fab, Fab', F(ab')₂, Fv, variable domain (e.g. VH or VL), diabody, minibody or monoclonal antibody. In a further embodiment, the antibody or fragment thereof is an scFv.

- 10 Antibodies of the invention can be of any class, e.g. IgG, IgA, IgM, IgE, IgD, or isotypes thereof, and can comprise a kappa or lambda light chain. In one embodiment, the antibody is an IgG antibody, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. In a further embodiment, the antibody may be in a format, such as an IgG format, that has been modified to confer desired properties, such as having the Fc mutated to reduce effector function, extend
- 15 half life, alter ADCC, or improve hinge stability. Such modifications are well known in the art.

- In one embodiment, the antibody or fragment thereof is human. Thus, the antibody or fragment thereof may be derived from a human immunoglobulin (Ig) sequence. The CDR, framework and/or constant region of the antibody (or fragment thereof) may be derived from a human Ig
- 20 sequence, in particular a human IgG sequence. The CDR, framework and/or constant region may be substantially identical for a human Ig sequence, in particular a human IgG sequence. An advantage of using human antibodies is that they are low or non-immunogenic in humans.

- An antibody or fragment thereof can also be chimeric, for example a mouse-human antibody
- 25 chimera.

- Alternatively, the antibody or fragment thereof is derived from a non-human species, such as a mouse. Such non-human antibodies can be modified to increase their similarity to antibody variants produced naturally in humans, thus the antibody or fragment thereof can be partially
- 30 or fully humanised. Therefore, in one embodiment, the antibody or fragment thereof is humanised.

Antibody sequences

- The isolated anti-V δ 1 antibodies, or fragments thereof, of the invention may be described with
- 35 reference to their CDR sequences.

According to one aspect of the invention, there is provided an isolated anti-V δ 1 antibody or fragment thereof, which comprises one or more of:

- a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25;
- 5 a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12; and/or
- a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.

- 10 According to one aspect of the invention, there is provided an isolated anti-V δ 1 antibody or fragment thereof, which comprises a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25. In one embodiment, the antibody or fragment thereof comprises a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3). In one
- 15 embodiment, the antibody or fragment thereof comprises a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.

- In one embodiment, the antibody or fragment thereof comprises a CDR3 comprising a sequence having at least 85%, 90%, 95%, 97%, 98% or 99% sequence identity with any one
- 20 of SEQ ID NOs: 2-25. In one embodiment, the antibody or fragment thereof comprises a CDR2 comprising a sequence having at least 85%, 90%, 95%, 97%, 98% or 99% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3). In one embodiment, the antibody or fragment thereof comprises a CDR1 comprising a sequence having at least 85%, 90%, 95%, 97%, 98% or 99% sequence identity with any one of SEQ ID
- 25 NOs: 38-61.

- In one embodiment, the antibody or fragment thereof comprises a CDR3 consisting of a sequence having at least 85%, 90%, 95%, 97%, 98% or 99% sequence identity with any one
- 30 of SEQ ID NOs: 2-25. In one embodiment, the antibody or fragment thereof comprises a CDR2 consisting of a sequence having at least 85%, 90%, 95%, 97%, 98% or 99% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3). In one embodiment, the antibody or fragment thereof comprises a CDR1 consisting of a sequence having at least 85%, 90%, 95%, 97%, 98% or 99% sequence identity with any one of SEQ ID
- 35 NOs: 38-61.

According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at

least 80% sequence identity with any one of SEQ ID NOs: 2-13 and/or a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25. According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence
5 having at least 80% sequence identity with any one of SEQ ID NOs: 2-13 and/or a VL region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25.

According to a particular aspect of the invention, there is provided an antibody or fragment
10 thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-7, in particular 2-6, such as 2, 3 or 4 and/or a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-19, in particular 14-18, such as 14, 15 or 16. According to another aspect of the invention, there is provided an antibody or fragment
15 thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-7, in particular 2-6, such as 2, 3 or 4 and/or a VL region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-19, in particular 14-18, such as 14, 15 or 16.

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According to a particular aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 8-13, in particular 8, 9, 10 or 11 and/or a VL region comprising a CDR3 comprising a sequence having at least 80% sequence
25 identity with any one of SEQ ID NOs: 20-25, in particular 20, 21, 22 or 23. According to another aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 8-13, in particular 8, 9, 10 or 11 and/or a VL region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ
30 ID NOs: 20-25, in particular 20, 21, 22 or 23.

According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 2-13 and/or a VL region comprising
35 a CDR3 comprising a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 14-25. According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence

having at least 90% sequence identity with any one of SEQ ID NOs: 2-13 and/or a VL region comprising a CDR3 consisting of a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 14-25.

- 5 According to a particular aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 2-7, in particular 2-6, such as 2, 3, 4 or 5 and/or a VL region comprising a CDR3 comprising a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 14-19, in particular 14-18, such as 14, 15, 16
10 or 17. According to another aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 2-7, in particular 2-6, such as 2, 3, 4 or 5 and/or a VL region comprising a CDR3 consisting of a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 14-19, in particular 14-18, such as 14, 15, 16
15 or 17.

- According to a particular aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 8, 9, 10 or 11 and/or a VL region
20 comprising a CDR3 comprising a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 20, 21, 22 or 23. According to another aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 90% sequence identity with any one of SEQ ID NOs: 8, 9, 10 or 11 and/or a VL region comprising a CDR3 consisting of a sequence having at least
25 90% sequence identity with any one of SEQ ID NOs: 20, 21, 22 or 23.

- According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 2-13 and/or a VL region comprising
30 a CDR3 comprising a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 14-25. According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 2-13 and/or a VL region comprising a CDR3 consisting of a sequence having at least 95% sequence identity with any
35 one of SEQ ID NOs: 14-25.

According to a particular aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 2-7, in particular 2-6, such as 2, 3, 4 or 5 and/or a VL region comprising a CDR3 comprising a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 14-19, in particular 14-18, such as 14, 15, 16 or 17. According to another aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 2-7, in particular 2-6, such as 2, 3, 4 or 5 and/or a VL region comprising a CDR3 consisting of a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 14-19, in particular 14-18, such as 14, 15, 16 or 17.

According to a particular aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 8, 9, 10 or 11 and/or a VL region comprising a CDR3 comprising a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 20, 21, 22 or 23. According to another aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 8, 9, 10 or 11 and/or a VL region comprising a CDR3 consisting of a sequence having at least 95% sequence identity with any one of SEQ ID NOs: 20, 21, 22 or 23.

According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13 and a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25. According to a further aspect of the invention, there is provided an antibody or fragment thereof, which comprises a VH region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13 and a VL region comprising a CDR3 consisting of a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25.

Embodiments which refer herein to "at least 80%" or "80% or greater", will be understood to include all values equal to or greater than 80%, such as 85%, 90%, 95%, 97%, 98%, 99% or 100% sequence identity. In one embodiment, the antibody or fragment of the invention comprises at least 85%, such as at least 90%, at least 95%, at least 97%, at least 98% or at least 99% sequence identity to the specified sequence.

Instead of percentage sequence identity, the embodiments may also be defined with one or more amino acid changes, for examples one or more additions, substitutions and/or deletions. In one embodiment, the sequence may comprise up to five amino acid changes, such as up to three amino acid changes, in particular up to two amino acid changes. In a further embodiment, the sequence may comprise up to five amino acid substitutions, such as up to three amino acid substitutions, in particular up to one or two amino acid substitutions. For example, CDR3 of the antibody or fragment thereof of the present invention comprises or more suitably consists of a sequence having no more than 2, more suitably no more than 1 substitution(s) compared to any one of SEQ ID NOs: 2-25.

Suitably any residues of CDR1, CDR2 or CDR3 differing from their corresponding residues in SEQ ID NO: 2-61 and SEQUENCES: A1-A12 are conservative substitutions with respect to their corresponding residues. For example, any residues of CDR3 differing from their corresponding residues in SEQ ID NOs: 2-25 are conservative substitutions with respect to their corresponding residues.

In one embodiment, the antibody or fragment thereof comprises:

- (i) a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13;
- (ii) a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37;
- (iii) a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-49;
- (iv) a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25;
- (v) a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12; and/or
- (vi) a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61.

In one embodiment, the antibody or fragment thereof comprises a heavy chain with:

- (i) a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13;
- (ii) a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37; and

(iii) a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-49.

In one embodiment, the antibody or fragment thereof comprises a light chain with:

- 5 (i) a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25;
- (ii) a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12; and
- 10 (iii) a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61.

In one embodiment, the antibody or fragment thereof comprises (or consists of) a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2, 3, 4, 5 or 6, such as 2, 3, 4 or 5, in particular 2, 3 or 4. In one
15 embodiment, the antibody or fragment thereof comprises (or consists of) a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26, 27, 28, 29 or 30, such as 26, 27, 28 or 29, in particular 26, 27 or 28. In one embodiment, the antibody or fragment thereof comprises (or consists of) a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any
20 one of SEQ ID NOs: 38, 39, 40, 41 or 42, such as 38, 39, 40 or 41, in particular 38, 39 or 40.

In one embodiment, the antibody or fragment thereof comprises (or consists of) a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 8, 9, 10 or 11. In one embodiment, the antibody or fragment thereof
25 comprises (or consists of) a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 32, 33, 34 or 35. In one embodiment, the antibody or fragment thereof comprises (or consists of) a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 44, 45, 46 or 47.

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In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 2, a CDR2 comprising a sequence of SEQ ID NO: 26, and a CDR1 comprising a sequence of SEQ ID NO: 38. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 2, the CDR2 consists of a sequence of SEQ ID NO: 26, and the CDR1 consists of a sequence of
35 SEQ ID NO: 38.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 3, a CDR2 comprising a sequence of SEQ ID NO: 27, and a CDR1 comprising a sequence of SEQ ID NO: 39. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 3, the CDR2 consists of a sequence of SEQ ID NO: 27, and the CDR1 consists of a sequence of SEQ ID NO: 39.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 4, a CDR2 comprising a sequence of SEQ ID NO: 28, and a CDR1 comprising a sequence of SEQ ID NO: 40. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 4, the CDR2 consists of a sequence of SEQ ID NO: 28, and the CDR1 consists of a sequence of SEQ ID NO: 40.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 5, a CDR2 comprising a sequence of SEQ ID NO: 29, and a CDR1 comprising a sequence of SEQ ID NO: 41. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 5, the CDR2 consists of a sequence of SEQ ID NO: 29, and the CDR1 consists of a sequence of SEQ ID NO: 41.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 6, a CDR2 comprising a sequence of SEQ ID NO: 30, and a CDR1 comprising a sequence of SEQ ID NO: 42. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 6, the CDR2 consists of a sequence of SEQ ID NO: 30, and the CDR1 consists of a sequence of SEQ ID NO: 42.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 8, a CDR2 comprising a sequence of SEQ ID NO: 32, and a CDR1 comprising a sequence of SEQ ID NO: 44. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 8, the CDR2 consists of a sequence of SEQ ID NO: 32, and the CDR1 consists of a sequence of SEQ ID NO: 44.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 9, a CDR2 comprising a sequence of SEQ ID NO: 33, and a CDR1 comprising a sequence of SEQ ID NO: 45. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 9, the CDR2 consists of a sequence of SEQ ID NO: 33, and the CDR1 consists of a sequence of SEQ ID NO: 45.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 10, a CDR2 sequence of SEQ ID NO: 34, and a CDR1 sequence of SEQ ID NO: 46. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 10, the CDR2 consists of a sequence of SEQ ID NO: 34, and the CDR1 consists of a sequence of SEQ ID NO: 46.

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In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 11, a CDR2 sequence of SEQ ID NO: 35, and a CDR1 sequence of SEQ ID NO: 47. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 11, the CDR2 consists of a sequence of SEQ ID NO: 35, and the CDR1 consists of a sequence of SEQ ID NO: 47.

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In one embodiment, the antibody or fragment thereof comprises (or consists of) a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25, such as SEQ ID NOs: 14, 15, 16, 17 or 18 such as 14, 15, 16 or 17, in particular 14, 15 or 16. In one embodiment, the antibody or fragment thereof comprises (or consists of) a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12 (of Table 3), such as SEQUENCES: A1, A2, A3, A4 or A5, such as A1, A2, A3 or A4, in particular A1, A2 or A3. In one embodiment, the antibody or fragment thereof comprises (or consists of) a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61, such as SEQ ID NOs: 50, 51, 52, 53 or 54, such as 50, 51, 52 or 53, in particular 50, 51 or 52.

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In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 14, a CDR2 comprising a sequence of SEQUENCE: A1, and a CDR1 comprising a sequence of SEQ ID NO: 50. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 14, the CDR2 consists of a sequence of SEQUENCE: A1, and the CDR1 consists of a sequence of SEQ ID NO: 50.

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In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 15, a CDR2 comprising a sequence of SEQUENCE: A2, and a CDR1 comprising a sequence of SEQ ID NO: 51. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 15, the CDR2 consists of a sequence of SEQUENCE: A2, and the CDR1 consists of a sequence of SEQ ID NO: 51.

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In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 16, a CDR2 comprising a sequence of SEQUENCE: A3, and a CDR1 comprising a sequence of SEQ ID NO: 52. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 16,

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the CDR2 consists of a sequence of SEQUENCE: A3, and the CDR1 consists of a sequence of SEQ ID NO: 52.

5 In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 17, a CDR2 comprising a sequence of SEQUENCE: A4, and a CDR1 comprising a sequence of SEQ ID NO: 53. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 17, the CDR2 consists of a sequence of SEQUENCE: A4, and the CDR1 consists of a sequence of SEQ ID NO: 53.

10 In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 18, a CDR2 comprising a sequence of SEQUENCE: A5, and a CDR1 comprising a sequence of SEQ ID NO: 54. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 18, the CDR2 consists of a sequence of SEQUENCE: A5, and the CDR1 consists of a sequence of SEQ ID NO: 54.

15 In one embodiment, the antibody or fragment thereof comprises (or consists of) a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 20, 21, 22 or 23. In one embodiment, the antibody or fragment thereof comprises (or consists of) a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A7, A8, A9 or A10. In one embodiment, the antibody or fragment thereof comprises (or consists of) a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 56, 57, 58 or 59.

25 In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 20, a CDR2 comprising a sequence of SEQUENCE: A7, and a CDR1 comprising a sequence of SEQ ID NO: 56. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 20, the CDR2 consists of a sequence of SEQUENCE: A7, and the CDR1 consists of a sequence of SEQ ID NO: 56.

30 In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 21, a CDR2 comprising a sequence of SEQUENCE: A8, and a CDR1 comprising a sequence of SEQ ID NO: 57. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 21, the CDR2 consists of a sequence of SEQUENCE: A8, and the CDR1 consists of a sequence of SEQ ID NO: 57.

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In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 22, a CDR2 comprising a sequence of SEQUENCE: A9, and a CDR1 comprising a sequence of SEQ ID NO: 58. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 22, the CDR2 consists of a sequence of SEQUENCE: A9, and the CDR1 consists of a sequence of SEQ ID NO: 58.

In one embodiment, the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 23, a CDR2 comprising a sequence of SEQUENCE: A10, and a CDR1 comprising a sequence of SEQ ID NO: 59. In one embodiment, the CDR3 consists of a sequence of SEQ ID NO: 23, the CDR2 consists of a sequence of SEQUENCE: A10, and the CDR1 consists of a sequence of SEQ ID NO: 59.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 2, a CDR2 comprising a sequence of SEQ ID NO: 26, a CDR1 comprising a sequence of SEQ ID NO: 38, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 14, a CDR2 comprising a sequence of SEQUENCE: A1, and a CDR1 comprising a sequence of SEQ ID NO: 50. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 2, the HCDR2 consists of a sequence of SEQ ID NO: 26, the HCDR1 consists of a sequence of SEQ ID NO: 38, the LCDR3 consists of a sequence of SEQ ID NO: 14, the LCDR2 consists of a sequence of SEQUENCE: A1, and the LCDR1 consists of a sequence of SEQ ID NO: 50.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 3, a CDR2 comprising a sequence of SEQ ID NO: 27, a CDR1 comprising a sequence of SEQ ID NO: 39, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 15, a CDR2 comprising a sequence of SEQUENCE: A2, and a CDR1 comprising a sequence of SEQ ID NO: 51. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 3, the HCDR2 consists of a sequence of SEQ ID NO: 27, the HCDR1 consists of a sequence of SEQ ID NO: 39, the LCDR3 consists of a sequence of SEQ ID NO: 15, the LCDR2 consists of a sequence of SEQUENCE: A2, and the LCDR1 consists of a sequence of SEQ ID NO: 51.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 4, a CDR2 comprising a sequence of SEQ ID NO: 28, a CDR1 comprising a sequence of SEQ ID NO: 40, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 16, a CDR2 comprising a sequence of SEQUENCE: A3, and a CDR1 comprising a sequence of SEQ ID NO: 52. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 4, the HCDR2 consists of a sequence of SEQ ID NO: 28, the HCDR1 consists of a sequence of

SEQ ID NO: 40, the LCDR3 consists of a sequence of SEQ ID NO: 16, the LCDR2 consists of a sequence of SEQUENCE: A3, and the LCDR1 consists of a sequence of SEQ ID NO: 52.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 5, a CDR2 comprising a sequence of SEQ ID NO: 29, a CDR1 comprising a sequence of SEQ ID NO: 41, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 17, a CDR2 comprising a sequence of SEQUENCE: A4, and a CDR1 comprising a sequence of SEQ ID NO: 53. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 5, the HCDR2 consists of a sequence of SEQ ID NO: 29, the HCDR1 consists of a sequence of SEQ ID NO: 41, the LCDR3 consists of a sequence of SEQ ID NO: 17, the LCDR2 consists of a sequence of SEQUENCE: A4, and the LCDR1 consists of a sequence of SEQ ID NO: 53.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 6, a CDR2 comprising a sequence of SEQ ID NO: 30, a CDR1 comprising a sequence of SEQ ID NO: 42, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 18, a CDR2 comprising a sequence of SEQUENCE: A5, and a CDR1 comprising a sequence of SEQ ID NO: 54. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 6, the HCDR2 consists of a sequence of SEQ ID NO: 30, the HCDR1 consists of a sequence of SEQ ID NO: 42, the LCDR3 consists of a sequence of SEQ ID NO: 18, the LCDR2 consists of a sequence of SEQUENCE: A5, and the LCDR1 consists of a sequence of SEQ ID NO: 54.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 7, a CDR2 comprising a sequence of SEQ ID NO: 31, a CDR1 comprising a sequence of SEQ ID NO: 43, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 19, a CDR2 comprising a sequence of SEQUENCE: A6, and a CDR1 comprising a sequence of SEQ ID NO: 55. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 7, the HCDR2 consists of a sequence of SEQ ID NO: 31, the HCDR1 consists of a sequence of SEQ ID NO: 43, the LCDR3 consists of a sequence of SEQ ID NO: 19, the LCDR2 consists of a sequence of SEQUENCE: A6, and the LCDR1 consists of a sequence of SEQ ID NO: 55.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 8, a CDR2 comprising a sequence of SEQ ID NO: 32, a CDR1 comprising a sequence of SEQ ID NO: 44, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 20, a CDR2 comprising a sequence of SEQUENCE: A7, and a CDR1 comprising a sequence of SEQ ID NO: 56. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 8, the HCDR2 consists of a sequence of SEQ ID NO: 32, the HCDR1 consists of a sequence of

SEQ ID NO: 44, the LCDR3 consists of a sequence of SEQ ID NO: 20, the LCDR2 consists of a sequence of SEQUENCE: A7, and the LCDR1 consists of a sequence of SEQ ID NO: 56.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 9, a CDR2 comprising a sequence of SEQ ID NO: 33, a CDR1 comprising a sequence of SEQ ID NO: 45, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 21, a CDR2 comprising a sequence of SEQUENCE: A8, and a CDR1 comprising a sequence of SEQ ID NO: 57. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 9, the HCDR2 consists of a sequence of SEQ ID NO: 33, the HCDR1 consists of a sequence of SEQ ID NO: 45, the LCDR3 consists of a sequence of SEQ ID NO: 21, the LCDR2 consists of a sequence of SEQUENCE: A8, and the LCDR1 consists of a sequence of SEQ ID NO: 57.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 10, a CDR2 comprising a sequence of SEQ ID NO: 34, a CDR1 comprising a sequence of SEQ ID NO: 46, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 22, a CDR2 comprising a sequence of SEQUENCE: A9, and a CDR1 comprising a sequence of SEQ ID NO: 58. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 10, the HCDR2 consists of a sequence of SEQ ID NO: 34, the HCDR1 consists of a sequence of SEQ ID NO: 46, the LCDR3 consists of a sequence of SEQ ID NO: 22, the LCDR2 consists of a sequence of SEQUENCE: A9, and the LCDR1 consists of a sequence of SEQ ID NO: 58.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 11, a CDR2 comprising a sequence of SEQ ID NO: 35, a CDR1 comprising a sequence of SEQ ID NO: 47, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 23, a CDR2 comprising a sequence of SEQUENCE: A10, and a CDR1 comprising a sequence of SEQ ID NO: 59. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 11, the HCDR2 consists of a sequence of SEQ ID NO: 35, the HCDR1 consists of a sequence of SEQ ID NO: 47, the LCDR3 consists of a sequence of SEQ ID NO: 23, the LCDR2 consists of a sequence of SEQUENCE: A10, and the LCDR1 consists of a sequence of SEQ ID NO: 59.

In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 12, a CDR2 comprising a sequence of SEQ ID NO: 36, a CDR1 comprising a sequence of SEQ ID NO: 48, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 24, a CDR2 comprising a sequence of SEQUENCE: A11, and a CDR1 comprising a sequence of SEQ ID NO: 60. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO: 12, the HCDR2 consists of a sequence of SEQ ID NO: 36, the HCDR1 consists of a sequence

of SEQ ID NO: 48, the LCDR3 consists of a sequence of SEQ ID NO: 24, the LCDR2 consists of a sequence of SEQUENCE: A11, and the LCDR1 consists of a sequence of SEQ ID NO: 60.

- 5 In one embodiment, the VH region comprises a CDR3 comprising a sequence of SEQ ID NO: 13, a CDR2 comprising a sequence of SEQ ID NO: 37, a CDR1 comprising a sequence of SEQ ID NO: 49, and the VL region comprises a CDR3 comprising a sequence of SEQ ID NO: 25, a CDR2 comprising a sequence of SEQUENCE: A12, and a CDR1 comprising a sequence of SEQ ID NO: 61. In one embodiment, the HCDR3 consists of a sequence of SEQ ID NO:
- 10 13, the HCDR2 consists of a sequence of SEQ ID NO: 37, the HCDR1 consists of a sequence of SEQ ID NO: 49, the LCDR3 consists of a sequence of SEQ ID NO: 25, the LCDR2 consists of a sequence of SEQUENCE: A12, and the LCDR1 consists of a sequence of SEQ ID NO: 61.
- 15 In one embodiment, the antibody or fragment thereof comprises one or more CDR sequences as described in **Table 3**. In a further embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1252_P01_C08 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1245_P01_E07 as described in **Table 3**. In an alternative
- 20 embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1245_P02_G04 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1245_P02_B07 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone
- 25 1251_P02_C05 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1139_P01_E04 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1245_P02_F07 as described in **Table 3**. In an alternative embodiment, the antibody or
- 30 fragment thereof comprises one or more (such as all) CDR sequences of clone 1245_P01_G06 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1245_P01_G09 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1138_P01_B09
- 35 as described in **Table 3**. In an alternative embodiment, the antibody or fragment thereof comprises one or more (such as all) CDR sequences of clone 1251_P02_G10 as described in **Table 3**.

Suitably the VH and VL regions recited above each comprise four framework regions (FR1-FR4). In one embodiment, the antibody or fragment thereof comprises a framework region (e.g. FR1, FR2, FR3 and/or FR4) comprising a sequence having at least 80% sequence identity with the framework region in any one of SEQ ID NOs: 62-85. In one embodiment, the antibody or fragment thereof comprises a framework region (e.g. FR1, FR2, FR3 and/or FR4) comprising a sequence having at least 90%, such as at least 95%, 97% or 99% sequence identity with the framework region in any one of SEQ ID NOs: 62-85. In one embodiment, the antibody or fragment thereof comprises a framework region (e.g. FR1, FR2, FR3 and/or FR4) comprising a sequence in any one of SEQ ID NOs: 62-85. In one embodiment, the antibody or fragment thereof comprises a framework region (e.g. FR1, FR2, FR3 and/or FR4) consisting of a sequence in any one of SEQ ID NOs: 62-85.

The antibodies described herein may be defined by their full light chain and/or heavy chain variable sequences. Therefore, according to a further aspect of the invention, there is provided an isolated anti-V δ 1 antibody or fragment thereof, which comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-85. According to a further aspect of the invention, there is provided an isolated anti-V δ 1 antibody or fragment thereof, which consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-85.

In one embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73. In one embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73. In a further embodiment, the VH region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62, 63, 64, 65 or 66, such as 62, 63, 64 or 65, in particular 62, 63 or 64. In a further embodiment, the VH region consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62, 63, 64, 65 or 66, such as 62, 63, 64 or 65, in particular 62, 63 or 64. In a further embodiment, the VH region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 68, 69, 70, 71, 72 or 73, such as 68, 69, 70 or 71. In a further embodiment, the VH region consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 68, 69, 70, 71, 72 or 73, such as 68, 69, 70 or 71.

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In one embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-

85. In one embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85. In a further embodiment, the VL region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74, 75, 76, 77 or 78, such as 74, 75, 76 or 77, in particular 74, 75, or 76. In a further embodiment, the VL region consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74, 75, 76, 77 or 78, such as 74, 75, 76 or 77, in particular 74, 75, or 76. In a further embodiment, the VL region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 80, 81, 82, 83, 84 or 85, such as 80, 81, 82 or 83. In a further embodiment, the VL region consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 80, 81, 82, 83, 84 or 85, such as 80, 81, 82 or 83.

In a further embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73 and a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85. In a further embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73 and a VL region consisting of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85.

In one embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 63 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 62 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 64 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 68 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 69 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 70 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 71 (1245_P01_G09).

In one embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 63 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of

SEQ ID NO: 62 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 64 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 68 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 69 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 70 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 71 (1245_P01_G09).

In one embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 75 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 74 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 76 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 80 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 81 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 82 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof comprises a VL region comprising an amino acid sequence of SEQ ID NO: 83 (1245_P01_G09).

In one embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 75 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 74 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 76 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 80 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 81 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 82 (1245_P01_G06). In an alternative embodiment, the antibody or fragment

thereof comprises a VL region consisting of an amino acid sequence of SEQ ID NO: 83 (1245_P01_G09).

- 5 In one embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 63 (1252_P01_C08) and a VL region comprising an amino acid sequence of SEQ ID NO: 75 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 62 (1245_P01_E07) and a VL region comprising an amino acid sequence of SEQ ID NO: 74 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof
- 10 comprises a VH region comprising an amino acid sequence of SEQ ID NO: 64 (1245_P02_G04) and a VL region comprising an amino acid sequence of SEQ ID NO: 76 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 68 (1139_P01_E04) and a VL region comprising an amino acid sequence of SEQ ID NO: 80 (1139_P01_E04). In an
- 15 alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 69 (1245_P02_F07) and a VL region comprising an amino acid sequence of SEQ ID NO: 81 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 70 (1245_P01_G06) and a VL region comprising an amino acid sequence of SEQ
- 20 ID NO: 82 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof comprises a VH region comprising an amino acid sequence of SEQ ID NO: 71 (1245_P01_G06) and a VL region comprising an amino acid sequence of SEQ ID NO: 83 (1245_P01_G09).
- 25 In one embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 63 (1252_P01_C08) and a VL region consisting of an amino acid sequence of SEQ ID NO: 75 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 62 (1245_P01_E07) and a VL region consisting of an amino acid sequence of
- 30 SEQ ID NO: 74 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 64 (1245_P02_G04) and a VL region consisting of an amino acid sequence of SEQ ID NO: 76 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 68 (1139_P01_E04) and a
- 35 VL region consisting of an amino acid sequence of SEQ ID NO: 80 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 69 (1245_P02_F07) and a VL region consisting of an

amino acid sequence of SEQ ID NO: 81 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 70 (1245_P01_G06) and a VL region consisting of an amino acid sequence of SEQ ID NO: 82 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof comprises a VH region consisting of an amino acid sequence of SEQ ID NO: 71 (1245_P01_G06) and a VL region consisting of an amino acid sequence of SEQ ID NO: 83 (1245_P01_G09).

For fragments comprising both the VH and VL regions, these may be associated either covalently (e.g. via disulphide bonds or a linker) or non-covalently. The antibody fragment described herein may comprise an scFv, *i.e.* a fragment comprising a VH region and a VL region joined by a linker. In one embodiment, the VH and VL region are joined by a (e.g. synthetic) polypeptide linker. The polypeptide linker may comprise a $(\text{Gly}_4\text{Ser})_n$ linker, where n = from 1 to 8, e.g. 2, 3, 4, 5 or 7. The polypeptide linker may comprise a $[(\text{Gly}_4\text{Ser})_n(\text{Gly}_3\text{AlaSer})_m]_p$ linker, where n = from 1 to 8, e.g. 2, 3, 4, 5 or 7, m = from 1 to 8, e.g. 0, 1, 2 or 3, and p = from 1 to 8, e.g. 1, 2 or 3. In a further embodiment, the linker comprises SEQ ID NO: 98. In a further embodiment, the linker consists of SEQ ID NO: 98.

In one embodiment, the antibody or fragment thereof comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 86-97. In a further embodiment, the antibody or fragment thereof comprises an amino acid sequence of any one of SEQ ID NOs: 86-97. In a yet further embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 87 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 86 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 88 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 92 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 93 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 94 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NO: 95 (1245_P01_G09).

In one embodiment, the antibody or fragment thereof consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 86-97. In a further embodiment, the antibody or fragment thereof consists of an amino acid sequence of any one of SEQ ID NOs: 86-97. In a yet further embodiment, the antibody or fragment thereof consists

of an amino acid sequence of SEQ ID NO: 87 (1252_P01_C08). In an alternative embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NO: 86 (1245_P01_E07). In an alternative embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NO: 88 (1245_P02_G04). In an alternative embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NO: 92 (1139_P01_E04). In an alternative embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NO: 93 (1245_P02_F07). In an alternative embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NO: 94 (1245_P01_G06). In an alternative embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NO: 95 (1245_P01_G09).

It will be understood by a person skilled in the art that scFv constructs may be designed and made inclusive of N-terminal and C-terminal modifications to aid with translation, purification and detection. For example, at the N-terminus of an scFv sequence, an additional methionine and/or alanine amino acid residue may be included ahead of the canonical VH sequences (e.g. starting QVQ or EVQ). At the C-terminus (*i.e.* C-terminal to the canonical VL domain sequence ending as per the IMGT definition), additional sequences may be included such as (i) a partial sequence of the constant domain and/or (ii) additional synthetic sequences inclusive of tags, such as His-tags and Flag-tags, to aid with purification and detection. In one embodiment, SEQ ID NO: 124 is added to the C-terminus of any one of SEQ ID NOs: 86, 88-90, 92-97. In one embodiment, SEQ ID NO: 125 is added to the C-terminus of any one of SEQ ID NOs: 86, 88-90, 92-97. In one embodiment, SEQ ID NO: 126 is added to the C-terminus of any one of SEQ ID NOs: 87 or 91. In one embodiment, SEQ ID NO: 127 is added to the C-terminus of any one of SEQ ID NOs: 87 or 91. It is well understood that said scFv N- or C-terminal sequences are optional and can be removed, modified or substituted if alternate scFv design, translation, purification or detection strategies are adopted.

As described herein, the antibodies may be in any format. In a preferred embodiment, the antibody is in an IgG1 format. Therefore, in one embodiment, the antibody or fragment thereof comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 111-122. In a further embodiment, the antibody or fragment thereof comprises an amino acid sequence of any one of SEQ ID NOs: 111-122. In a yet further embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NOs: 111-116, such as SEQ ID NOs: 111-113 and 116. In a yet further embodiment, the antibody or fragment thereof comprises an amino acid sequence of SEQ ID NOs: 117-122, such as SEQ ID NOs: 117-120. In a yet further embodiment, the antibody or fragment thereof comprises an

amino acid sequence of SEQ ID NOs: 111, 112, 116-120, such as SEQ ID NOs: 111, 112 or 116, or SEQ ID NOs: 117-120.

5 In one embodiment, the antibody or fragment thereof consists of an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 111-122. In a further embodiment, the antibody or fragment thereof consists of an amino acid sequence of any one of SEQ ID NOs: 111-122. In a yet further embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NOs: 111-116, such as SEQ ID NOs: 111-113 and 116. In a yet further embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NOs: 117-122, such as SEQ ID NOs: 117-120. In a yet further embodiment, the antibody or fragment thereof consists of an amino acid sequence of SEQ ID NOs: 111, 112, 116-120, such as SEQ ID NOs: 111, 112 or 116, or SEQ ID NOs: 117-120.

15 In one embodiment, the antibody binds to the same, or essentially the same, epitope as, or competes with, an antibody or fragment thereof as defined herein. One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-V δ 1 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-V δ 1 antibody of the invention, the reference antibody is allowed to bind to a V δ 1 protein or peptide under saturating conditions. 20 Next, the ability of a test antibody to bind to the V δ 1 chain is assessed. If the test antibody is able to bind to V δ 1 following saturation binding with the reference anti-V δ 1 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-V δ 1 antibody. On the other hand, if the test antibody is not able to bind to the V δ 1 chain following saturation binding with the reference anti-V δ 1 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-V δ 1 antibody of the invention. 25

The present invention also includes anti-V δ 1 antibodies that compete for binding to V δ 1 with an antibody or fragment thereof as defined herein, or an antibody having the CDR sequences of any of the exemplary antibodies described herein. For example, competitive assays can be 30 performed with the antibody of the present invention in order to determine what proteins, antibodies, and other antagonists compete for binding to the V δ 1 chain with the antibody of the present invention and/or share the epitope. These assays are readily known to those of skill in the art; they evaluate competition between antagonists or ligands for a limited number of binding sites on a protein, *e.g.* V δ 1. The antibody (or fragment thereof) is immobilized or 35 insolubilized before or after the competition and the sample bound to the V δ 1 chain is separated from the unbound sample, for example, by decanting (where the antibody was pre-insolubilized) or by centrifuging (where the antibody was precipitated after the competitive

reaction). Also, the competitive binding may be determined by whether the function is altered by the binding or lack of binding of the antibody to the protein, e.g. whether the antibody molecule inhibits or potentiates the enzymatic activity of, for example, a label. ELISA and other functional assays may be used, as known in the art and described herein.

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Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the target antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay. Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the target antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

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Additional routine experimentation (e.g. peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

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In some embodiments, the antibody or fragment thereof contains a modified effector function through alteration to the sugars linked to Asn 297 (EU numbering scheme). In a further said modification, Asn 297 is not fucosylated or exhibits reduced fucosylation (i.e., a defucosylated antibody or a non-fucosylated antibody). Fucosylation includes the addition of the sugar fucose to a molecule, for example, the attachment of fucose to N-glycans, O-glycans and glycolipids. Accordingly, in a defucosylated antibody, fucose is not attached to the carbohydrate chains of the constant region. The antibody may be modified to prevent or inhibit fucosylation of the antibody. Typically, glycosylation modifications involve expressing said antibody or fragment thereof in a host cell containing alternate glycosylation processing capabilities either through targeted engineering or through targeted or serendipitous host or clone selection (e.g. see Example 13). These and other effector modifications are discussed further in recent reviews such as by Xinhua Wang et al. (2018) Protein & Cell 9: 63-73 and by Pereira et al. (2018) mAbs 10(5): 693-711 and which are hereby incorporated

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Antibody sequence modifications

The antibodies and fragments thereof may be modified using known methods. Sequence modifications to antibody molecules described herein can be readily incorporate by those skilled in the art. The following examples are non-limiting.

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During antibody discovery and sequence recovery from phage libraries, desired antibody variable domains may be re-formatted into full length IgG by sub-cloning. To accelerate the process, variable domains are often transferred using restriction enzymes. These unique restriction sites may introduce additional/alternate amino acids and away from the canonical sequence (such canonical sequences may be found, for example, in the international ImMunoGeneTics [IMGT] information system, see <http://www.imgt.org>). These may be introduced as kappa or lambda light chain sequence modifications.

Kappa light chain modifications

15 The variable kappa light chain variable sequences may be cloned using restriction sites (e.g. Nhe1-Not1) during re-formatting into full length IgG. More specifically, at the kappa light chain N-terminus, an additional Ala-Ser sequence was introduced to support cloning. Preferably, this additional AS sequence is then removed during further development such to generate the canonical N-terminal sequence. Hence, in one embodiment, kappa light chain containing
20 antibodies described herein do not contain an AS sequence at their N-termini, i.e. SEQ ID NOs: 74, 76-78 and 80-85 do not comprise the initial AS sequence. In a further embodiment, SEQ ID NOs: 74 and 76-78 do not comprise the initial AS sequence. It will be understood that this embodiment also applies to other sequences included herein which contain this sequence (e.g. SEQ ID NOs: 86, 88-90 and 92-97).

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Additional amino acid changes may be made to support cloning. For example, for the antibodies described herein, at the kappa light-chain variable-domain/constant domain border a valine-to-alanine change was introduced to support cloning. This resulted in a kappa constant domain modification. Specifically, this results in the constant domain beginning
30 RTAAAPS (from a NotI restriction site). Preferably, this sequence can be modified during further development to generate the canonical kappa light-chain constant regions which start with RTVAAPS. Hence, in one embodiment kappa light chain containing antibodies described herein contain a constant domain stating with the sequence RTV. Therefore, in one embodiment, sequence RTAAAPS of SEQ ID NOs: 111-114 and 117-122 is replaced with
35 sequence RTVAAPS. For example, see Example 13 and SEQ ID NOs: 129, 130.

Lambda light chain modifications

Similar to the kappa example above, the lambda light chain variable domains may also be cloned by introducing restriction sites (e.g. Nhe1-Not1) during re-formatting into full length IgG. More specifically, at the lambda light chain N-terminus, an additional Ala-Ser sequence may be introduced to support cloning. Preferably, this additional AS sequence is then removed during further development such to generate the canonical N-terminal sequence. Hence, in one embodiment, lambda light chain containing antibodies described herein do not contain an AS sequence at their N-termini *i.e.* SEQ ID NOs: 75 and 79 do not comprise the initial AS sequence. It will be understood that this embodiment also applies to other sequences included herein which contain this sequence (e.g. SEQ ID NOs: 87, 91, 115 and 116). In one embodiment, SEQ ID NO: 75 does not contain the initial six residues, *i.e.* the ASSYEL sequence is removed.

As another example, for the antibodies described herein at the lambda light-chain variable-domain/constant domain border a lysine-to-alanine sequence change was introduced to support cloning. This resulted in a lambda constant domain modification. Specifically, this results in the constant domain beginning with GQPAAAPS (from a NotI restriction site). Preferably, this sequence can be modified during further development such to generate the canonical lambda light constant region which starts GQPKAAPS. Hence, in one embodiment, lambda light chain containing antibodies described herein contain a constant domain starting with the sequence GQPK. Therefore, in one embodiment, sequence GQPAAAPS of SEQ ID NO: 115 or 116 is replaced with sequence GQPKAAPS.

Heavy chain modifications

Typically, human variable heavy chain sequences start with either the basic glutamine (Q) or acidic glutamate (E). However, both such sequences are then known to convert to the acidic amino acid residue, pyro-glutamate (pE). The Q to pE conversion results in a charge change to the antibody, whilst an E to pE conversion does not change the charge of the antibody. Hence to avoid a variable charge-change over time one option is to modify a starting heavy chain sequence from Q to E in the first instance. Hence, in one embodiment, the heavy chain of antibody described herein contains a Q to E modification at the N-terminus. In particular, the initial residue of SEQ ID NOs: 62, 64 and/or 67-71 may be modified from Q to E. It will be understood that this embodiment also applies to other sequences included herein which contain this sequence (e.g. SEQ ID NOs: 86, 88, 91-97 and 111, 112, 115, 117-120). For example, see Example 13 and SEQ ID NOs: 129, 130.

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Furthermore, the C-terminus of the IgG1 constant domain ends with PGK. However, the terminal basic lysine (K) is then often cleaved during expression (e.g. in CHO cells). This in

turn results in charge change to the antibody through varied loss of the C-terminal lysine residue. Therefore, one option is to remove the lysine in the first instance resulting in a uniform and consistent heavy chain C-terminus sequence ending in PG. Hence, in one embodiment, the heavy chain of an antibody described herein has the terminal K removed from its C-terminus. In particular, the antibody of the invention may comprise any one of SEQ ID NOs: 111-122 where the terminal lysine residue has been removed. For example, see SEQ ID NO: 141.

Optional allotype modifications

During antibody discovery, specific human allotypes may be employed. Optionally, the antibodies can be switched to differing human allotypes during development. By way of non-limiting example, for the kappa chain there are three human allotypes designated Km1, Km1,2 and Km3 which define three Km alleles (using allotype numbering): Km1 correlates with valine 153 (IMGT V45.1) and leucine 191 (IMGT L101); Km1,2 correlates with alanine 153 (IMGT A45.1) and leucine 191 (IMGT L101); and Km3 correlates with alanine 153 (IMGT A45.1) and valine 191 (IMGT V101). Optionally, one can therefore modify a sequence from one allotype to another by standard cloning approaches. For example, a L191V (IMGT L101V) change will convert a Km1,2 allotype to a Km3 allotype. For further reference on such allotypes see Jefferis and Lefranc (2009) *MAbs* 1(4):332-8, which is herein incorporated by reference.

Hence in one embodiment an antibody described herein contains amino acid substitutions derived from another human allotype of the same gene. In a further embodiment, the antibody contains a L191V (IMGT L101V) substitution to the kappa chain to convert the c-domain from a km1,2 to a km3 allotype. For example, see Example 13 and SEQ ID NOs: 129, 130.

Antibodies targeted to epitopes

Provided herein are antibodies (or fragments thereof) which bind to an epitope of the V δ 1 chain of a $\gamma\delta$ TCR. Such binding may optionally have an effect on $\gamma\delta$ TCR activity, such as activation or inhibition.

In one embodiment, the epitope may be an activating epitope of a $\gamma\delta$ T cell. An "activating" epitope can include, for example, stimulating a TCR function, such as cell degranulation, TCR downregulation, cytotoxicity, proliferation, mobilisation, increased survival or resistance to exhaustion, intracellular signaling, cytokine or growth factor secretion, phenotypic change, or a change in gene expression. For example, the binding of the activating epitope may stimulate expansion (*i.e.* proliferation) of the $\gamma\delta$ T cell population, preferably the V δ 1+ T cell population. Accordingly, these antibodies can be used to modulate $\gamma\delta$ T cell activation, and, thereby, to

modulate the immune response. Therefore, in one embodiment, binding of the activating epitope downregulates the $\gamma\delta$ TCR. In an additional or alternative embodiment, binding of the activating epitope activates degranulation of the $\gamma\delta$ T cell. In a further additional or alternative embodiment, binding of the activating epitope activates $\gamma\delta$ T cell killing.

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Alternatively, the antibodies (or fragments thereof) may have a blocking effect by prevention of the binding or interaction of another antibody or molecule. In one embodiment, the present invention provides isolated antibodies or fragments thereof that block V δ 1 and prevent TCR binding (e.g. through steric hinderance). By blocking V δ 1, the antibody may prevent TCR
10 activation and/or signalling. The epitope may be an inhibitory epitope of a $\gamma\delta$ T cell. An "inhibitory" epitope can include, for example, blocking TCR function, thereby inhibiting TCR activation.

In one embodiment, the present invention provides isolated antibodies or fragments thereof
15 that bind to V δ 1 but do not activate $\gamma\delta$ T cells (i.e., a non-activating or a non-inhibitory or a neutral binding antibody or fragment thereof) and do not prevent TCR binding, for example via its 'private' CDR3 encoded paratope. By binding to V δ 1, but not inhibiting or activating $\gamma\delta$ T cell activity, the neutral binding antibody or fragment thereof can be used, in some embodiments, to co-localize another molecule. For example, the neutral-binding V δ 1 antibody
20 may be conjugated to a therapeutic moiety, such as a cytotoxin or a chemotherapeutic agent. Such conjugates may be referred to as immunoconjugates. The antibody may be linked to the cytotoxin, radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, toxin, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunoconjugates include antibody drug conjugates and antibody-toxin
25 fusion proteins. In one embodiment, the agent may be a second different antibody to V δ 1. In certain embodiments, the antibody may be conjugated to an agent specific for a tumor cell or a virally infected cell. The type of therapeutic moiety that may be conjugated to the anti-V δ 1 antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. In one embodiment, the agent may be a second antibody, or fragment
30 thereof, that binds to a molecule other than V δ 1.

The epitope is preferably comprised of at least one extracellular, soluble, hydrophilic, external or cytoplasmic portion of the V δ 1 chain of a $\gamma\delta$ TCR.

35 In particular, the epitope does not comprise an epitope found in a hypervariable region of the V δ 1 chain of the $\gamma\delta$ TCR, in particular CDR3 of the V δ 1 chain. In a preferred embodiment, the epitope is within the non-variable region of the V δ 1 chain of the $\gamma\delta$ TCR. It will be appreciated

that such binding allows for the unique recognition of the V δ 1 chain without the restriction to the sequences of the TCR which are highly variable (in particular CDR3). Various $\gamma\delta$ TCR complexes which recognise MHC-like peptides or antigen may be recognised in this way, solely by presence of the V δ 1 chain. As such, it will be appreciated that any V δ 1 chain-comprising $\gamma\delta$ TCR may be recognised using the antibodies or fragments thereof as defined herein, irrespective of the specificity of the $\gamma\delta$ TCR. In one embodiment, the epitope comprises one or more amino acid residues within amino acid regions 1-24 and/or 35-90 of SEQ ID NO: 1, e.g. the portions of the V δ 1 chain which are not part of the CDR1 and/or CDR3 sequences. In one embodiment, the epitope does not comprise amino acid residues within amino acid region 91-105 (CDR3) of SEQ ID NO: 1.

In a similar manner to the well characterised $\alpha\beta$ T cells, $\gamma\delta$ T cells utilize a distinct set of somatically rearranged variable (V), diversity (D), joining (J), and constant (C) genes, although $\gamma\delta$ T cells contain fewer V, D, and J segments than $\alpha\beta$ T cells. In one embodiment, the epitope bound by the antibodies (or fragments thereof) does not comprise an epitope found in the J region of the V δ 1 chain (e.g. one of the four J regions encoded in the human delta one chain germline: SEQ ID NO:152 (J1*0) or 153 (J2*0) or 154 (J3*0) or 155 (J4*0)) or in the C-region of the V δ 1 chain (e.g. SEQ ID NO:156 (C1*0) which contains the C-terminal juxtamembrane/transmembrane regions). In one embodiment, the epitope bound by the antibodies (or fragments thereof) does not comprise an epitope found in the N-terminal leader sequence of the V δ 1 chain (e.g. SEQ ID NO:150). The antibody or fragment may therefore only bind in the V region of the V δ 1 chain (e.g. SEQ ID NO: 151). Thus, in one embodiment, the epitope consists of an epitope in the V region of the $\gamma\delta$ TCR (e.g. amino acid residues 1-90 of SEQ ID NO: 1).

Reference to the epitope are made in relation to the V δ 1 sequence derived from the sequence described in Luoma *et al.* (2013) *Immunity* 39: 1032-1042, and RCSB Protein Data Bank entries: 4MNH and 3OMZ, shown as SEQ ID NO: 1:

AQKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKS
GRYSVNFKKAASVALTISALQLEDSAKYFCALGESLTRADKLIFGKGTRVTVEPNIQNPDP
VYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNK
SDFACANAFNNSIIPEDTFFPSPESS (SEQ ID NO: 1)

SEQ ID NO: 1 represents a soluble TCR comprising a V region (also referred to as the variable domain), a D region, a J region and a TCR constant region. The V region comprises amino acid residues 1-90, the D region comprises amino acid residues 91-104, the J region

comprises amino acid residues 105-115 and the constant region comprises amino acid residues 116-209. Within the V region, CDR1 is defined as amino acid residues 25-34 of SEQ ID NO: 1, CDR2 is defined as amino acid residues 50-54 of SEQ ID NO: 1, and CDR3 is defined as amino acid residues 93-104 of SEQ ID NO: 1 (Xu *et al.*, PNAS USA 108(6):2414-2419 (2011)).

Therefore, according to an aspect of the invention, there is provided an isolated antibody or fragment thereof, which binds to an epitope of a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR) comprising one or more amino acid residues within amino acid regions:

- (i) 3-20 of SEQ ID NO: 1; and/or
- (ii) 37-77 of SEQ ID NO: 1.

In a further embodiment, antibodies or fragments thereof additionally recognize the polymorphic V region comprising amino acid residues 1-90 epitope of SEQ ID NO:128. Hence, amino acids 1-90 of SEQ ID NO:1 and the polymorphic germline variant sequence (amino acids 1-90 SEQ ID NO:128) may be considered interchangeable when defining epitopes described herein. Studies presented herein have demonstrated antibodies of the invention can recognize both variants of this germline sequence. By way of example, where it is stated that antibodies or fragments thereof as defined herein recognize epitopes comprising one or more amino acid residues within amino acid regions 1-24 and/or 35-90 of SEQ ID NO:1 this also refers to the same regions of SEQ ID NO:128; specifically amino acid regions 1-24 and/or 35-90 of SEQ ID NO:128.

In one embodiment, antibodies or fragments thereof recognize one or more amino acid residues within amino acid regions 1-90 of SEQ ID NO:1 and the equivalently located amino acids of regions 1-90 in SEQ ID NO:128. More specifically, in one embodiment antibodies or fragments thereof as defined herein recognize a human germline epitope wherein said germline encodes either an alanine (A) or valine (V) at position 71 of SEQ ID NO:1.

In one embodiment, the epitope comprises one or more, such as two, three, four, five, six, seven, eight, nine, ten or more amino acid residues within the described regions.

In a further embodiment, the epitope comprises one or more (such as 5 or more, such as 10 or more) amino acid residues within amino acid region 3-20 of SEQ ID NO: 1. In an alternative embodiment, the epitope comprises one or more (such as 5 or more, such as 10 or more) amino acid residues within amino acid region 37-77 of SEQ ID NO: 1 (such as amino acid region 50-54). In a yet further embodiment, the epitope comprises one or more (such as 5 or

more, such as 10 or more) amino acid residues within amino acid region 3-20 (such as 5-20 or 3-17) and one or more (such as 5 or more, such as 10 or more) amino acid residues within amino acid region 37-77 (such as 62-77 or 62-69) of SEQ ID NO: 1.

- 5 It will be further understood that said antibody (or fragment thereof) does not need to bind to all amino acids within the defined range. Such epitopes may be referred to as linear epitopes. For example, an antibody which binds to an epitope comprising amino acid residues within amino acid region 5-20 of SEQ ID NO: 1 may only bind with one or more of the amino acid residues in said range, e.g. the amino acid residues at each end of the range (*i.e.* amino acids
10 5 and 20), optionally including amino acids within the range (*i.e.* amino acids 5, 9, 16 and 20).

- In one embodiment, the epitope comprises at least one of amino acid residues 3, 5, 9, 10, 12, 16, 17, 20, 37, 42, 50, 53, 59, 62, 64, 68, 69, 72 or 77 of SEQ ID NO: 1. In further embodiments, the epitope comprises one, two, three, four, five, six, seven, eight, nine, ten, eleven or twelve
15 amino acids selected from amino acid residues 3, 5, 9, 10, 12, 16, 17, 20, 37, 42, 50, 53, 59, 62, 64, 68, 69, 72 or 77 of SEQ ID NO: 1.

In one embodiment, the epitope comprises one or more amino acid residues within the following amino acid regions of SEQ ID NO: 1 (or SEQ ID NO:128, as described above):

- 20 (i) 3-17;
(ii) 5-20;
(iii) 37-53;
(iv) 50-64;
(v) 59-72;
25 (vi) 59-77;
(vii) 62-69; and/or
(viii) 62-77.

- In a further embodiment, the epitope comprises one or more amino acid residues within amino
30 acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69, of SEQ ID NO: 1. In a further embodiment, the epitope consists of one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69, of SEQ ID NO: 1.

- 35 In a further embodiment, the epitope comprises amino acid residues: 3, 5, 9, 10, 12, 16, 17, 62, 64, 68 and 69 of SEQ ID NO: 1, or suitably consists of amino acid residues: 3, 5, 9, 10, 12, 16, 17, 62, 64, 68 and 69 of SEQ ID NO: 1. In a further embodiment, the epitope comprises

amino acid residues: 5, 9, 16, 20, 62, 64, 72 and 77 of SEQ ID NO: 1, or suitably consists of amino acid residues: 5, 9, 16, 20, 62, 64, 72 and 77 of SEQ ID NO: 1. In yet further embodiment, the epitope comprises the amino acid residues: 37, 42, 50, 53, 59, 64, 68, 69, 72, 73 and 77 of SEQ ID NO: 1, or suitably consists of amino acid residues: 37, 42, 50, 53, 59, 64, 68, 69, 72, 73 and 77 of SEQ ID NO: 1. In a further embodiment, the epitope comprises the amino acid residues: 50, 53, 59, 62 and 64 of SEQ ID NO: 1, or suitably consists of amino acid residues: 50, 53, 59, 62 and 64 of SEQ ID NO: 1. In a further embodiment, the epitope comprises amino acid residues: 59, 60, 68 and 72 of SEQ ID NO: 1, or suitably consists of amino acid residues: 59, 60, 68 and 72 of SEQ ID NO: 1.

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In one embodiment, the epitope comprises one or more amino acid residues within amino acid regions 5-20 and/or 62-77 of SEQ ID NO: 1. In a further embodiment, the epitope consists of one or more amino acid residues within amino acid regions 5-20 and 62-77 of SEQ ID NO: 1. In an alternative further embodiment, the epitope comprises one or more amino acid residues within amino acid regions 5-20 or 62-77 of SEQ ID NO: 1. Antibodies or fragments thereof having such epitopes may have some or all of the sequences of 1245_P01_E07, or such antibodies or fragments thereof may be derived from 1245_P01_E07. For example, antibodies or fragments thereof having one or more CDR sequences of 1245_P01_E07 or one or both of the VH and VL sequences of 1245_P01_E07 may bind such epitopes.

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In one embodiment, the epitope comprises one or more amino acid residues within amino acid region 50-64 of SEQ ID NO: 1. In a further embodiment, the epitope consists of one or more amino acid residues within amino acid region 50-64 of SEQ ID NO: 1. Antibodies or fragments thereof having such epitopes may have some or all of the sequences of 1252_P01_C08, or such antibodies or fragments thereof may be derived from 1252_P01_C08. For example, antibodies or fragments thereof having one or more CDR sequences of 1252_P01_C08 or one or both of the VH and VL sequences of 1252_P01_C08 may bind such epitopes.

In one embodiment, the epitope comprises one or more amino acid residues within amino acid regions 37-53 and/or 59-77 of SEQ ID NO: 1. In a further embodiment, the epitope consists of one or more amino acid residues within amino acid regions 37-53 and 59-77 of SEQ ID NO: 1. In an alternative further embodiment, the epitope comprises one or more amino acid residues within amino acid regions 37-53 or 59-77 of SEQ ID NO: 1. Antibodies or fragments thereof having such epitopes may have some or all of the sequences of 1245_P02_G04, or such antibodies or fragments thereof may be derived from 1245_P02_G04. For example, antibodies or fragments thereof having one or more CDR sequences of 1245_P02_G04 or one or both of the VH and VL sequences of 1245_P02_G04 may bind such epitopes.

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In one embodiment, the epitope comprises one or more amino acid residues within amino acid region 59-72 of SEQ ID NO: 1. In a further embodiment, the epitope consists of one or more amino acid residues within amino acid region 59-72 of SEQ ID NO: 1. Antibodies or fragments thereof having such epitopes may have some or all of the sequences of 1251_P02_C05, or such antibodies or fragments thereof may be derived from 1251_P02_C05. For example, antibodies or fragments thereof having one or more CDR sequences of 1251_P02_C05 or one or both of the VH and VL sequences of 1251_P02_C05 may bind such epitopes.

10 In one embodiment, the epitope does not comprise amino acid residues within amino acid region 11-21 of SEQ ID NO: 1. In one embodiment, the epitope does not comprise amino acid residues within amino acid region 21-28 of SEQ ID NO: 1. In one embodiment, the epitope does not comprise amino acid residues within amino acid region 59 and 60 of SEQ ID NO: 1. In one embodiment, the epitope does not comprise amino acid residues within amino acid region 67-82 of SEQ ID NO: 1.

In one embodiment, the epitope is not the same epitope bound by a commercially available anti-V δ 1 antibody, such as TS-1 or TS8.2. As described in WO2017197347, binding of TS-1 and TS8.2 to soluble TCRs was detected when the δ 1 chain included V δ 1 J1 and V δ 1 J2 sequences but not to the V δ 1 J3 chain, indicating that the binding of TS-1 and TS8.2 involved critical residues in the delta J1 and delta J2 region.

References to "within" herein include the extremities of the define range. For example, "within amino acid regions 5-20" refers to all of amino acid residues from and including residue 5 up to and including residue 20.

Various techniques are known in the art to establish which epitope is bound by an antibody. Exemplary techniques include, for example, routine cross-blocking assays, alanine scanning mutational analysis, peptide blot analysis, peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed. Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry (as described in Example 9). In general terms, the hydrogen/deuterium exchange method involves deuterium-labelling the protein of interest, followed by binding the antibody to the deuterium-labelled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-

exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labelled residues which correspond to the specific amino acids with which the antibody interacts.

Antibody binding

The antibody or fragment thereof of the invention may bind to the V δ 1 chain of a $\gamma\delta$ TCR with a binding affinity (KD) as measured by surface plasmon resonance of less than 1.5×10^{-7} M (*i.e.* 150 nM). In a preferred embodiment, the KD is less than 1.5×10^{-7} M (*i.e.* 150 nM). In a further embodiment, the KD is 1.3×10^{-7} M (*i.e.* 130 nM) or less, such as 1.0×10^{-7} M (*i.e.* 100 nM) or less. In a yet further embodiment, the KD is less than 5.0×10^{-8} M (*i.e.* 50 nM), such as less than 4.0×10^{-8} M (*i.e.* 40 nM), less than 3.0×10^{-8} M (*i.e.* 30 nM) or less than 2.0×10^{-8} M (*i.e.* 20 nM). For example, according to some aspects, there is provided a human anti-V δ 1 antibody which binds to the V δ 1 chain of a $\gamma\delta$ TCR with a binding affinity (KD) as measured by surface plasmon resonance of less than 1.5×10^{-7} M (*i.e.* 150 nM).

In one aspect of the invention, there is provided an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR with a binding affinity (KD) as measured by surface plasmon resonance of less than 4.0×10^{-8} M (*i.e.* 40 nM), less than 3.0×10^{-8} M (*i.e.* 30 nM) or less than 2.0×10^{-8} M (*i.e.* 20 nM).

In one embodiment, the binding affinity of the antibody or fragment thereof is established by coating the antibody or fragment thereof directly or indirectly (*e.g.* by capture with an anti-human IgG Fc) onto the surface of a sensor (*e.g.* an amine high capacity chip or equivalent), wherein the target bound by the antibody or fragment thereof (*i.e.* the V δ 1 chain of a $\gamma\delta$ TCR) is flowed over the chip to detect binding. Suitably, a MASS-2 instrument (which may also be referred to as Sierra SPR-32) is used at 25 °C in PBS + 0.02 % Tween 20 running buffer at 30 μ l/min.

Described herein are other assays which may be used to define antibody function. For example, the antibody or fragment thereof described herein may be assessed by $\gamma\delta$ TCR engagement, *e.g.* measuring downregulation of the $\gamma\delta$ TCR upon antibody binding. Surface expression of the $\gamma\delta$ TCR following application of the antibody or fragment thereof (optionally presented on the surface of a cell) can be measured, *e.g.* by flow cytometry. The antibody or fragment thereof described herein may also be assessed by measuring $\gamma\delta$ T cell

degranulation. For example, expression of CD107a, a marker for cell degranulation, can be measured following application of the antibody or fragment thereof (optionally presented on the surface of a cell) to $\gamma\delta$ T cells, e.g. by flow cytometry. The antibody or fragment thereof described herein may also be assessed by measuring $\gamma\delta$ T cell killing activity (to test if the antibody has an effect on the killing activity of the $\gamma\delta$ T cell). For example, target cells may be incubated with $\gamma\delta$ T cells in the presence of the antibody or fragment thereof (optionally presented on the surface of a cell). Following incubation, the culture may be stained with a cell viability dye to distinguish between live and dead target cells. The proportion of dead cells can then be measured, e.g. by flow cytometry.

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Medicaments for modulating $\gamma\delta$ T cells

The anti-V δ 1 antibodies or fragments thereof as described herein may be used to modulate delta variable 1 chain (V δ 1) T cells in a patient *in situ* (i.e. *in vivo*).

15 Modulation of V δ 1 T cells may include:

- expansion of the V δ 1 T cells, e.g. by selectively increasing the number of V δ 1 T cells or promotion of survival of V δ 1 T cells;
- stimulation of the V δ 1 T cells, e.g. by increasing V δ 1 T cell potency, i.e. increasing target cell killing;
- prevention of V δ 1 T cell exhaustion, e.g. by increasing persistence of the V δ 1 T cells;
- degranulation of V δ 1 T cells;
- immunosuppression of the V δ 1 T cells, e.g. by downregulation of V δ 1 TCR cell surface expression, i.e. by causing V δ 1 TCR internalisation or reduced expression of V δ 1 TCR protein, or blocking the V δ 1 TCR from binding;
- reducing V δ 1 T cell number, e.g. by inhibition of V δ 1 T cell proliferation or by inducing V δ 1 T cell death (i.e. killing V δ 1 T cells).

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Medicaments that modulate immune cell markers on V δ 1+ cells

30 The antibody or fragment thereof may modulate immune cell markers of V δ 1+ cells upon administration to a patient.

An antibody or fragment thereof described herein may also be assessed for its suitability for its therapeutic use by measuring $\gamma\delta$ T modulation. For example, by measuring a change in the levels of CD25 or CD69 or CD107a present on a V δ 1+ T-cell or cells in a model system. Such markers are often used as markers of lymphocyte modulation (e.g. proliferation or degranulation) and can be measured following application of an antibody or fragment thereof

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as described herein, e.g. by flow cytometry. Surprisingly, during such assessments (e.g. see Examples 7, 17, 18) it was observed that antibodies as described herein conferred measurably higher levels of CD25 or CD69 or CD107a levels on target V δ 1+ T-cells. Optionally, the change in phenotype of a V δ 1+ cell or population thereof tested in the model system can then be compared to the change in phenotype when an alternative comparator antibody is applied (e.g. OKT-3, TS8.2, etc.) to said equivalent $\gamma\delta$ T cells.

Hence in one aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR for therapeutic use comprising administering the antibody or fragment thereof to a cell population comprising V δ 1+ cells and determining the effect on the level of CD25 and/or CD69 and/or CD107a on the surface of the V δ 1+ cells. The effect on the level of CD25, CD69 and/or CD107a may be determined/measured over a period of time. It will be understood that the effect can be measured in comparison to the level of CD25 and/or CD69 and/or CD107a on the surface of the V δ 1+ cell when said antibody is not applied to said cell over the same period of time. In a further aspect of the invention there is provided a method of selecting or characterizing or comparing the antibodies or fragments thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by adding said antibodies to a cell population comprising V δ 1+ cells and then measuring the level (or expression) of CD25 or CD69 or CD107a on the surface of said V δ 1+ cells.

Medicaments that modulate growth properties or numbers of V δ 1+ cells

The antibody or fragment thereof may modulate the growth properties of V δ 1+ cells upon administration to a patient. For example, the antibody or fragment thereof may expand V δ 1+ cells.

An alternate approach to measuring $\gamma\delta$ T proliferation may include measuring the change in relative number of V δ 1+ cells over time when applying an antibody or fragment thereof as described herein to model systems containing said cells. Surprisingly, during such assessments it was observed that antibodies as described herein were able to measurably increase the number of said V δ 1+ T-cells (e.g. see Example 10, 17 and 18). Optionally this change in number can then be compared to the change in number observed when an alternative comparator antibody is applied (e.g. anti-OKT3) to said model systems.

Hence in another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the

antibody or fragment thereof to a cell population comprising V δ 1+ cells and determining the effect on the number of V δ 1+ cells in the population. The effect on cell number can be determined/measured over a period of time. It will be understood that the effect can be measured in comparison to the effect on cell numbers observed when said antibody is not applied to the cell population for the same period of time. In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by applying said antibodies to a cell population comprising V δ 1+ cells and then measuring the number of said cells over time.

Medicaments that modulate the proliferative capacity and numbers of V δ 1+ cells

An ideal therapeutic antibody or fragment thereof as described herein which binds to the V δ 1 chain of a $\gamma\delta$ TCR may be one that is capable of enhancing the proliferation of V δ 1+ cells *in vivo*. Such antibodies can then be employed as medicaments designed to specifically increase the V δ 1+ cell number in a subject or patient. For example:

Cancer:

Relative increases in the numbers of V δ 1+ cells have been reported as a positive prognostic indicator associated with improved outcomes for many cancer (for example see Gentles *et al* (2015) Nature Immunology 21: 938-945; Wu et al. (2019) Sci. Trans. Med. 11(513): eaax9364; Catellani et al. (2007) Blood 109(5): 2078-2085). In one embodiment, presented herein is a medicament capable of increasing the relative or absolute numbers of V δ 1+ cells *in situ* within in a cancer patient.

Pathogenic/Parasitic/Viral Infections:

V δ 1+ cell enrichment is observed during host defense against numerous acquired pathogenic/parasitic/viral infections. For recent general review see Zhao *et al.* (2018) Immunol. Res. 2018:5081634. Furthermore, increased numbers V δ 1+ are also considered protective against a variety of DNA and RNA viral infections. For example, increased numbers are also considered protective during CMV infections associated with allogeneic transplants (see van Dorp et al. (2011) Biology of Blood and Marrow Transplantation 17(2): S217). Additionally, V δ + cell numbers increase in patients with coronavirus infection (Poccia et al. (2006) J. Infect. Dis. 193(9): 1244-1249).

In another embodiment, presented herein is a medicament capable of increasing the relative or absolute numbers of V δ 1+ cells in a subject or patient harboring a pathogenic infection.

Stem Cell Transplant:

Increased numbers of V δ 1+ cells have also been associated with less disease relapse, fewer viral infections, higher overall and disease-free survival and favorable clinical outcomes in general during hematopoietic stem cell transplant (for example see Aruda et al. (2019) Blood 3(21): 3436–3448 and see Godder et al. (2007) Bone Marrow Transplantation 39: 751-757). Hence another embodiment, presented herein is a medicament capable of increasing the relative or absolute numbers of V δ 1+ cells in a subject as part of a treatment regimen supporting a stem cell transplant.

Consequently, a medicament capable of preferentially or specifically increasing the numbers of V δ 1+ cells *in-situ* is highly desirable.

Medicaments that maintain or induce or increase V δ 1+ cell cytokine secretion

Cytokines are a large group of proteins, peptides or glycoproteins that are secreted by specific cells of immune system. They are a category of signaling molecules that mediate and regulate immunity, inflammation, and hematopoiesis. A number of cytokines have been implicated in ameliorating signs and symptoms of disease through either direct or indirect modulation of the tumour and cellular microenvironment, autoimmune tissue and associated microenvironment, or virally infected tissue or cellular environment. Exemplar pro-inflammatory cytokines include tumour necrosis factor-alpha (TNF α) and Interferon-gamma (IFN γ).

However, many such cytokines exhibit unfavourable toxicity when dosed systemically. For example, whilst TNF α can induce the haemorrhagic necrosis of transplanted tumours, and has been reported to exert synergic anti-tumour effects when combined with other chemotherapeutic drugs, various clinical trials with systemic recombinant human TNF α (rhTNF α) have highlighted significant dose limiting side effects inclusive of hypotension, rigors, phlebitis, thrombocytopenia, leucopenia and hepatotoxicity, fever, fatigue, nausea/vomiting, malaise and weakness, headache, chest tightness, low back pain, diarrhoea and shortness of breath.

Use of recombinant IFN γ also faces similar systemic toxicity challenges. For example, whilst in a cancer setting IFN γ can exert favorable pleiotropic effects including MHC class I and II upregulation to stimulate anti-tumour immunity, increasing T-cell infiltration, conferring anti-angiogenesis effects, inducing chemokine / cytokine secretion, and exerting direct cancer cell anti-proliferative effects, adverse side-effects are also observed. These include fever,

headache, chills, fatigue, diarrhoea, nausea, vomiting, anorexia, transient increases in hepatic transaminase, and transient decreases in granulocyte and leucocyte counts.

For a recent review on both the potential and limitation of systemic recombinant TNF α and IFN γ see Shen et al. (2018) Cell Prolif. 51(4): e12441.

Hence there is a need for more *in situ* controlled, more localized, more tissue or cell specific production of such cytokines. For example, more controlled expression or induction of pro-inflammatory cytokines is proposed as one approach whereby “cold” tumours can be turned “hot”. Hot tumours are also sometimes termed “T-cell-inflamed” because of an increase in the number or density of CD45+ T-cells also observed. See Bonaventura et al. (2019) Front. Immunol. 10: 168 for a recent review.

For such reasons, an ideal therapeutic antibody or fragment thereof as described herein which binds to the V δ 1 chain of a $\gamma\delta$ TCR may be one that can maintain or enhance or induce the secretion of cytokines in V δ 1+ cells *in vivo*. Such antibodies can then be employed as medicaments designed to specifically increase or induce cytokines in a subject or patient and in a more localized, less systemic manner and one which better correlates with the distribution of V δ 1+cells in said subject or patient.

Remarkably, when antibodies as described herein which bind to the the V δ 1 chain of a $\gamma\delta$ TCR are applied to V δ 1+ cells, a significantly higher level of secreted cytokines are observed. More specifically, and as a non-limiting example, a significant higher level of TNF α and IFN γ is observed. See Example 15.

Hence in another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the antibody or fragment thereof to a cell population comprising V δ 1+ cells and determining the amount of at least one cytokine produced by the cell population. The amount of cytokine produced can be determined/measured over a period of time and optionally compared to the amount observed when said antibody is not applied to the cell population for the same period of time. In one embodiment, the observed level of cytokine produced when the antibody is administered to the cell population is more than about 10%, more than about 20%, more than about 30%, more than about 50%, more than about 100%, more than about 150%, more than about 200%, more than about 250%, more than about 300%, more than about 350%, more than about 400%, more than about 450%, more than about 500%, more than about 1000%, relative to the level of cytokine produced when the antibody is not applied. In a further aspect

of the invention, the cytokine is a pro-Inflammatory cytokine. In a further aspect of the invention, the cytokine is the TNF- α cytokine. In a further aspect of the invention, is IFN- γ cytokine.

- 5 In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by applying said antibodies to a cell population comprising V δ 1+ cells and then measuring the level of at least one cytokine generated. In a further aspect of the invention the cytokine measured is TNF- α cytokine and/or IFN- γ cytokine.

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In a further aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR by applying said antibody or fragment thereof to a cell population comprising V δ 1+ cells and measuring the effect of the antibody on modulating a colder or cold tumour to become a hotter or hot tumour by
15 determining the quantity of proinflammatory cytokines produced and/or the number or density of CD45+ T-cells present in the tumour or tumour microenvironment.

Medicaments that maintain or induce or increase V δ 1+ cell Granzyme B activity

- 20 Granzyme B is a serine protease commonly found in the granules of natural killer cells (NK cells) and cytotoxic T cells. It is secreted by these cells along with the pore forming protein perforin to mediate apoptosis in target cells, such as diseased cells.

When V δ 1+ cells are incubated in co-cultures with target diseased cells (such as cancer cells)
25 in model systems, levels of Granzyme B levels and activity can be measured in the target diseased cells ahead of lysis. Remarkably when an antibody or fragment thereof as described herein which binds to the V δ 1 chain of a $\gamma\delta$ TCR is then applied to such co-cultures of V δ 1+ cells and cancer cells in such model systems, higher Granzyme B levels and activity are then observed in the diseased cancer cells ahead of cell death (see Example 16).

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Hence in another aspect of the invention, there is provided a method for assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the antibody or fragment thereof to a co-culture comprising V δ 1+ cells and diseased cells (such as cancer cells) and measuring the effect on the amount of Granzyme B produced by the
35 diseased cells in the co-culture. The amount of cytokine produced can be determined/measured over a period of time and optionally compared to the amount observed when said antibody is not applied to said co-cultures for the same period of time. In one

embodiment, the level of Granzyme B measured when said antibody is applied to said co-culture is more than about 10%, more than about 20%, more than about 30%, more than about 40%, more than about 50%, more than about 70%, more than about 80%, more than about 90%, more than about 100%, more than about 200%, relative to the Granzyme B level
5 observed when said antibody is not applied.

In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by applying said antibodies to a co-culture comprising V δ 1+ cells and diseased cells
10 and then measuring the quantity or activity of Granzyme B in the diseased cell.

Medicaments that expand polyclonal V δ 1+ cell populations

An ideal antibody medicament may also be one designed to ensure the expanding V δ 1+ cells
15 do not become too clonally focused at the hypervariable CDR3 sequence level. Hence an ideal antibody medicament may be designed such to avoid inducing proliferation V δ 1+ cells by binding to specific or 'private' δ 1+ CDR3 sequence paratopes. Rather, the antibody may bind via conserved germline sequences present on all V δ 1+ T cell receptors and in a gamma-chain independent manner, rather than bind to sequences presented only a sub-set of V δ 1+
20 cells.

Hence an ideal antibody medicament may stimulate the expansion V δ 1+ cells to generate a plurality of V δ 1+ cells containing a mixture of CDR3 sequences. This in turn would result in an *in vivo* expanded heterogenous polyclonal population of V δ 1+ cells displaying different CDR3
25 sequences on delta variable 1 chains. Remarkably, during analysis of expanded V δ 1+ cell populations generated by a method of adding an antibody or fragment thereof as described herein to a starting population of immune cells containing V δ 1+ cells, extensive polyclonality is observed by RNAseq based methodologies designed to sequence through the CDR3 hypervariable regions of RNA extracted (see Example 10).

30 Accordingly in one aspect, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the antibody or fragment thereof to a cell population comprising V δ 1+ cells and determining the polyclonality of the expanded V δ 1+ cells. It is desirable for an antibody medicament to generate an
35 expanded polyclonal population containing a plurality of V δ 1+ CDR3 sequences. Polyclonality can be determined using methods known in the art, such as by nucleic acid sequencing

approaches capable of analysing the V δ 1 chain hypervariable CDR3 content of said V δ 1+ cells.

Medicaments that expand polyclonal V δ 1+ cells for extended periods of time

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An ideal antibody medicament may be able to enhance or promote or stimulate the proliferation of primary V δ 1+ cells without exhausting such cells *in vivo*. For example, and by way of comparison, anti-CD3 medicaments such as OKT3 (e.g. Muronomab), whilst capable of expanding CD3 positive T-cells may also exhaust or induce anergy. To assess the capacity of antibodies as described herein and which bind to the V δ 1 chain of a $\gamma\delta$ TCR to drive continued cell division of viable V δ 1+ cells, longer term proliferation studies were undertaken. Remarkably these studies revealed that antibodies as described herein and which bind to the V δ 1 chain of a $\gamma\delta$ TCR are capable of driving cell division/proliferation of viable and still functionally cytotoxic V δ 1+ cells for over 40 days (see Example 10).

15

In one embodiment, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising applying the antibody or fragment thereof to a cell population and monitoring the length of time V δ 1+ cell division occurs. Ideally, the antibody is capable of stimulating V δ 1+ cell division for a period of 5 to 60 days, such as at least 7 to 45 days, 7 to 21 days, or 7 to 18 days.

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In a further embodiment, there is provided an antibody or fragment thereof as described herein which binds to the V δ 1 chain of a $\gamma\delta$ TCR and which when administered to a patient is capable of stimulating V δ 1+ cell division to increase the number by at least 2-fold in number, at least 5-fold in number, at least 10-fold in number, at least 25-fold in number, at least 50-fold in number, at least 60-fold in number, at least 70-fold in number, at least 80-fold in number, at least 90-fold in number, at least 100-fold in number, at least 200-fold in number, at least 300-fold in number, at least 400-fold in number, at least 500-fold in number, at least 600-fold in number, at least 1,000-fold in number.

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In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by applying said antibodies to V δ 1+ cells or mixed cell population containing V δ 1+ cells and then measuring V δ 1+ cell numbers over time.

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Medicaments that modulate non-V δ 1+ immune cells through targeting V δ 1+ immune cells

An antibody or fragment thereof as described herein may also be assessed by measuring V δ 1+ cell mediated modulation of other immune cells. For example, a change observed in a non- $\gamma\delta$ T cell 'fraction' can be measured following application of an antibody or fragment thereof as described herein to a model system comprising mixed population of immune cells such as one comprising human tissue $\alpha\beta$ cells and $\gamma\delta$ T cells. Further, the effect on non- $\gamma\delta$ cell types in said models can be measured by flow cytometry. For example, by measuring the relative change in numbers of CD8+ $\alpha\beta$ T cells upon addition of an antibody or fragment thereof as described herein to mixed cultures comprising $\gamma\delta$ T cells and non- $\gamma\delta$ T cells. Optionally, the observed change in number or phenotype of a non- $\gamma\delta$ T-cell CD8+ lymphocyte population can then be compared to the change in number when an alternative comparator antibody is applied (e.g. OKT-3) to said mixed population.

Hence in another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the antibody or fragment thereof to a mixed population of immune cells or tissues comprising V δ 1+ cells and V δ 1-negative immune cells and measuring the effect on the V δ 1-negative immune cells. The effect can be determined/measured over a period of time and optionally compared to the effect observed in V δ 1-negative cells when said antibody is not applied for the same period of time. The effect may be measured as a change in the number of V δ 1-negative immune cells. For example, the antibody may increase the number V δ 1-negative immune cells by more than about 10%, more than about 20%, more than about 30%, more than about 40%, more than about 50%, more than about 70%, more than about 80%, more than about 90%, more than about 100%, more than about 500%, relative to the levels observed when said antibody is not applied.

In a further aspect of the invention the modulated V δ 1-negative cell is a CD45+ cell. In a further aspect of the invention the modulated cell is a $\alpha\beta$ T-cell. In a further aspect of the invention the modulated $\alpha\beta$ + cell is CD8+ lymphocyte. In a further aspect of the invention the modulated $\alpha\beta$ T-cell, or population thereof, exhibits evidence of enhanced cell division. In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by administering said antibodies to a population of mixed immune cells comprising V δ 1+ cells and V δ 1-negative immune cells and then measuring an effect conferred on the V δ 1-negative cell population by V δ 1+ cells modulated by said antibodies or fragments thereof.

Optionally, and during "V δ 1+ cell mediated immune system modulation" as conferred by an antibody or fragment thereof as described herein, a concomitant increase in V δ 1+ cell number

is also observed. And whilst not being bound by this theory, it is possible that said increase in V δ 1+ cell number may be causal in driving the concomitant expansion of co-present V δ 1-negative immune cells, such as $\alpha\beta$ T-cells. An alternate hypothesis may be that antibody-induced cytokine secretions from the V δ 1+ T cells stimulate the expansion of V δ 1-negative immune cells.

In a further aspect of the invention the observed increase in $\alpha\beta$ + CD8+ lymphocyte population is compared to a comparator antibody such as OKT3 antibody or alternate anti-V δ 1 antibody. In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR by applying said antibodies to a population of mixed immune cells comprising V δ 1+ T-cells and $\alpha\beta$ T-cells and then measuring the numbers of CD8+ $\alpha\beta$ + T-cells lymphocytes over time.

15 Medicaments that modulate Tumour Infiltrating Lymphocytes (TILs)

An antibody or fragment thereof as described herein may also be assessed by measuring the effect conferred on tumour-infiltrating populations (TILs) in model systems. Surprisingly (see Example 18) during such assessments antibodies as described herein measurably modulated TIL populations in human tumours. For example, a change in either the number or phenotype of $\gamma\delta$ + lymphocyte TIL population or the non- $\gamma\delta$ lymphocyte TIL population is measured following application of an antibody or fragment thereof as described herein to a human tumour such as a human renal cell carcinoma. Optionally, the observed change in number or phenotype of either the $\gamma\delta$ + lymphocyte TIL population or non- $\gamma\delta$ lymphocyte TIL population can then be compared to the change observed when an alternative comparator antibody is applied (e.g. OKT-3) to said model system.

Hence in another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the antibody or fragment thereof to TILs located in, or derived from, a human tumour and determining the effect on the number of TILs. The effect can be determined/measured over a period of time and optionally compared to the TIL number observed when said antibody is not applied over the same period of time. The effect may be an increase in the number of TILs. For example, the antibody may increase the number of TILs more than about 10%, more than about 20%, more than about 30%, more than about 40%, more than about 50%, more than about 70%, more than about 80%, more than about 90%, more than about 100% relative to the number of TILs observed when said antibody is not applied. In a further aspect, the TILs

in which the number observed are $\gamma\delta$ + lymphocyte TIL cells and/or non- $\gamma\delta$ lymphocyte TIL cells.

5 In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR cells antibodies by applying said antibodies to TIL or TILs located in or derived from a human tumour and then measuring the change in number of TIL or TILs cells over a period of time.

10 Medicaments that modulate human V δ 1+ cytotoxicity

An antibody or fragment thereof as described herein may also be assessed by measuring the conferred effect on V δ 1+ mediated cell cytotoxicity. Surprisingly, during such assessments of antibodies as described herein (e.g. see Example 19) measurably enhanced V δ 1+ mediated
15 cell cytotoxicity was observed. For example, a reduction in the number of cancer cells or an increase in the number of killed cancer cells is observed following application of an antibody or fragment thereof to a model system comprising a mixed culture comprising V δ 1+ cells and said cancer cells. Optionally, the reduction in the number of cancer cells or the increase in the number of killed cancer cells can then be compared to the outcome when an alternative
20 comparator antibody is applied (e.g. OKT-3) to said model systems.

Hence in another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising applying the antibody or fragment thereof to a mixed population of cells comprising V δ 1+ cells and cancer cells and
25 measuring the cytotoxicity of the V δ 1+ cells towards the cancer cells. The cytotoxicity may be measured by an increase in the number of dead cancer cells over a period of time, optionally compared to the number of dead cancer cells observed when said antibody is not applied to the mixed population of cells over the same period of time. For example, the observed increase in dead cells when said antibody is applied may be more than about 10%, by more
30 than about 20%, by more than about 30%, by more than about 40%, by more than about 50%, more than about 70%, more than about 80%, more than about 90%, more than about 100%, more than about 200%, more than about 500%, relative to the number of dead cells observed when said antibody is not applied.

35 In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR cells by adding said antibodies to said population of mixed immune cells comprising

human V δ 1+ cells and cancer cells and then measuring an increase in dead cancer cells overtime.

Medicaments that modulate V δ 1+ cell target-to-effector cell ratios (T:E Ratios)

5

An antibody or fragment thereof as described herein may also be assessed by measuring how said antibodies enhance V δ 1+ mediated cancer cell cytotoxicity by determining the target cell to effector cell ratio wherein the 50% of the target cells (EC50) are killed in a model system to assess said antibodies as potential medicaments. For example, mixed cultures comprising target cancer cells with human V δ 1+ effector cells. Surprisingly, during such assessments (e.g. see Example 19) antibodies as described herein favourably modify the EC50 T:E ratio in model systems. Such modifications can be measured as numbers of V δ 1+ cells required to observe 50% killing of cancer cells over a set time. This can also be reported as change or as fold-improvements or as percent-improvements in cytotoxicity towards said cancer cells. Optionally, the T:E ratio conferred by antibodies of this invention can then be compared to the T:E ratios when an alternative comparator antibody is applied (e.g. OKT-3) to said model systems.

Hence in another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising applying the antibody or fragment thereof to a mixed population of cells comprising human V δ 1+ cells and cancer cells and measuring the number of V δ 1+ cells required to kill 50% of the cancer cells. This may be measured relative to the number V δ 1+ cells required to kill 50% of cancer cells without application of said antibody, optionally over the same period of time. For example, the reduction in the number of V δ 1+ cells required to kill 50% of the cancer cells when said antibody is applied may be greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, greater than about 50%, greater than about 70%, greater than about 80%, greater than about 90%, greater than about 100%, greater about 200%, greater than about 500%, relative to the number of V δ 1+ cells required to kill 50% of the cancer cells when said antibody is not applied.

In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain by adding said antibodies to said population of cells comprising V δ 1+ cells plus cancer cells and then measuring the numbers of V δ 1+ cells required to kill 50% of the cancer cells.

Medicaments which enhance Vδ1+ cell EC50 cytotoxicity

An alternate way to measure the observed enhanced cytotoxicity of human Vδ1+ cells or population thereof is to measure the number of cells required to kill 50% of the cancer cells over a set period of time in condition A (such as starting control) and compare this to the number of cells required to kill 50% of the cancer cells over a set period of time in condition B (such as upon application of antibody of the invention as described herein).

Whilst it is recognized that there are a variety of ways to measure such parameters, to aid in understanding, the following non-limiting hypothetical example will be outlined:

Hypothetically, effector cell cytotoxicity enhancement can be measured as follows:- In condition A (control treatment) it is observed that 1000 Vδ1+ cells are required to kill 50% of the cancer cells over a set period of time (e.g. 5 hours). In condition B (e.g. application of antibody of the invention described herein) it is observed that 500 Vδ1+ cells were required to kill 50% of the cancer cell over the same period of time. Hence in this example, the application of an antibody has enhanced the cytotoxicity of the Vδ1+ cell population by 200%:

$$(1000/500) \times 100 = 200\%$$

For example (see example 19), surprisingly such percentage enhancements have been observed for antibodies of the invention as described herein.

In a further aspect of the invention there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the Vδ1 chain of a γδ TCR by adding said antibodies to said population of mixed immune cells comprising Vδ1+ cells and cancer cells and determining the relative or percent-change in cytotoxicity versus an equivalent or control experiment wherein there is no application of said antibody to said mixture of cells.

Medicaments which enhance Vδ1+ cells diseased-cell specificity whilst sparing healthy cells

Another approach to assess antibodies or fragments thereof as described herein is to measure how said antibodies modulate diseased-cell specific cytotoxicity. Surprisingly during such studies, it was discovered such antibodies can specifically enhance the Vδ1+ cell specific killing of diseased-cells such as cancer cells (e.g. see Example 19) whilst sparing healthy or non-diseased cells. Ideal antibody medicaments administered to a patient to ameliorate a symptom of cancer will confer enhanced cytotoxicity specifically towards diseased cells whilst

sparing healthy cells. And medicaments which enhance effector cell cytotoxicity specifically towards diseased cells, such as cancer cells, can be said to exhibit an enhanced therapeutic index (TI) over medicaments which do not selectively enhance effector cell cytotoxicity specifically towards said diseased cells. The therapeutic index is also referred to as therapeutic ratio and is a quantitative measurement of the relative safety of a drug. It is a comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxicity e.g. by conferring undesirable death in related or relevant healthy cell populations. An antibody or fragment thereof as described herein may be assessed by measuring its ability to change or to enhance or to fold-improve V δ 1+ cell capacity to selectively kill diseased cells over and above healthy cells in model systems. For example, said model systems may comprise V δ 1+ effector cells, cancer cells, and control cells (such as healthy cells). Optionally, the fold-improvement in selective diseased-cell killing conferred by antibodies of the invention can then be compared to the fold-improvement observed when an alternative comparator antibody is applied (e.g. OKT-3) to said model systems.

The diseased-cell specificity and diseased-cell specificity-enhancement of V δ 1+ cells can be measured in cultures comprising V δ 1+ cells, diseased cells, and healthy cells. For example, V δ 1+ specificity towards diseased cells can be measured by observing the number of cancer cells killed by the V δ 1+ cells and then comparing the number of healthy cells killed by the V δ 1+ cells. Such comparisons can be controlled by including equivalent numbers of diseased cells and healthy cells in a model system also containing V δ 1+ cells e.g. "tricultures". Alternative comparison methodology can also be considered - for example when analytical or equipment limitations reduce the ability to distinguish and track all three cell types or more in parallel in a single assay (inclusive of V δ 1+ cells, diseased cells, and non-diseased cells). In said instances, comparing V δ 1+ cell cytotoxicity towards diseased cells in one experiment and then comparing V δ 1+ cell cytotoxicity towards healthy cells in a separate equivalent experiment offers an alternate approach to such studies.

In another aspect of the invention, there is provided a method of assessing an antibody or fragment thereof which binds to the V δ 1 chain of a $\gamma\delta$ TCR comprising administering the antibody or fragment thereof to a cell population comprising V δ 1+ cells and target cells and measuring the cell cytotoxic specificity towards the target cells. In one embodiment, the cell cytotoxicity specificity to a first target cell type can be compared to the cytotoxicity observed towards a second target cell type, therefore the method may be repeated using different target cell types. In a further aspect of the invention the first target cell type is a diseased cell and the second target cell type is a control cell such as a healthy cell or a cell with a different disease to the first target cell type.

In a further aspect of the invention there is provided a method for selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR wherein the effect conferred by said antibody on V δ 1+ cell cytotoxicity towards (i) a first cell type and (ii) a second cell type is measured and compared. In a further aspect of the invention an antibody is thereby selected which enhances the specific cytotoxicity towards the first cell type more so than towards the second cell type. In a further aspect of the invention the first cell type is a diseased-cell and the second cell type is a healthy cell.

As described herein, the antibodies or fragments thereof used in the assays may be presented on a surface, for example the surface of a cell, such as a cell comprising an Fc receptor. For example, the antibodies or fragments thereof may be presented on the surface of THP-1 cells, such as TIB-202™ cells (available from American Type Culture Collection (ATCC)). Alternatively, the antibodies or fragments thereof may be used directly in the assays.

In such functional assays, output may be measured by calculating the half maximal concentration, also referred to as "EC50" or "effective concentration at 50 percent". The term "IC50" refers to the inhibitory concentration. Both EC50 and IC50 may be measured using methods known in the art, such as flow cytometry methods. In some instances, EC50 and IC50 are the same value or can be considered equivalent. For example, the effective concentration (EC) of effector cells required to inhibit (e.g. kill) 50% of a certain cell type may also be considered the 50% inhibitory concentration (IC). For the avoidance of doubt, the values of EC50 in the present application are provided using IgG1 formatted antibody when referring to an antibody. Such values can be easily converted based on the molecular weight of the antibody format for equivalent values as follows:

$$(\mu\text{g/ml}) / (\text{MW in kDa}) = \mu\text{M}$$

The EC50 for downregulation of the $\gamma\delta$ TCR upon antibody (or fragment) binding may be less than 0.50 $\mu\text{g/ml}$, such as less than 0.40 $\mu\text{g/ml}$, 0.30 $\mu\text{g/ml}$, 0.20 $\mu\text{g/ml}$, 0.15 $\mu\text{g/ml}$, 0.10 $\mu\text{g/ml}$, 0.06 $\mu\text{g/ml}$ or 0.05 $\mu\text{g/ml}$. In a preferred embodiment, the EC50 for downregulation of the $\gamma\delta$ TCR upon antibody (or fragment) binding is less than 0.10 $\mu\text{g/ml}$. In particular, the EC50 for downregulation of the $\gamma\delta$ TCR upon antibody (or fragment) binding may be less than 0.06 $\mu\text{g/ml}$, such as less than 0.05 $\mu\text{g/ml}$, 0.04 $\mu\text{g/ml}$ or 0.03 $\mu\text{g/ml}$. In particular, said EC50 values are when the antibody is measured in an IgG1 format. For example, the EC50 $\gamma\delta$ TCR downregulation value can be measured using flow cytometry (e.g. as described in the assay of Example 6).

The EC50 for $\gamma\delta$ T cell degranulation upon antibody (or fragment) binding may be less than 0.050 $\mu\text{g/ml}$, such as less than 0.040 $\mu\text{g/ml}$, 0.030 $\mu\text{g/ml}$, 0.020 $\mu\text{g/ml}$, 0.015 $\mu\text{g/ml}$, 0.010 $\mu\text{g/ml}$ or 0.008 $\mu\text{g/ml}$. In particular, the EC50 for $\gamma\delta$ T cell degranulation upon antibody (or fragment) binding may be less than 0.005 $\mu\text{g/ml}$, such as less than 0.002 $\mu\text{g/ml}$. In a preferred embodiment, the EC50 for $\gamma\delta$ T cell degranulation upon antibody (or fragment) binding is less than 0.007 $\mu\text{g/ml}$. In particular, said EC50 values are when the antibody is measured in an IgG1 format. For example, the $\gamma\delta$ T cell degranulation EC50 value can be measured by detecting CD107a expression (*i.e.* a marker of cell degranulation) using flow cytometry (*e.g.* as described in the assay of Example 7). In one embodiment, CD107a expression is measured using an anti-CD107a antibody, such as anti-human CD107a BV421 (clone H4A3) (BD Biosciences).

The EC50 for $\gamma\delta$ T cell killing upon the antibody (or fragment) binding may be less than 0.50 $\mu\text{g/ml}$, such as less than 0.40 $\mu\text{g/ml}$, 0.30 $\mu\text{g/ml}$, 0.20 $\mu\text{g/ml}$, 0.15 $\mu\text{g/ml}$, 0.10 $\mu\text{g/ml}$ or 0.07 $\mu\text{g/ml}$. In a preferred embodiment, the EC50 for $\gamma\delta$ T cell killing upon the antibody (or fragment) binding is less than 0.10 $\mu\text{g/ml}$. In particular, the EC50 for $\gamma\delta$ T cell killing upon the antibody (or fragment) binding may be less than 0.060 $\mu\text{g/ml}$, such as less than 0.055 $\mu\text{g/ml}$, in particular less than 0.020 $\mu\text{g/ml}$. In particular, said EC50 values are when the antibody is measured in an IgG1 format. For example, the EC50 $\gamma\delta$ T cell killing value can be measured by detecting proportion of dead cells (*i.e.* using a cell viability dye) using flow cytometry following incubation of the antibody, $\gamma\delta$ T cell and target cells (*e.g.* as described in the assay of Example 8). In one embodiment, death of the target cell is measured using a cell viability dye is Viability Dye eFluor™ 520 (ThermoFisher).

In the assays described in these aspects, the antibody or fragment thereof may be presented on the surface of a cell, such as a THP-1 cell, for example TIB-202™ (ATCC). The THP-1 cells are optionally labelled with a dye, such as CellTracker™ Orange CMTMR (ThermoFisher).

30

Immunoconjugates

The antibodies or fragments thereof of the present invention may be conjugated to a therapeutic moiety, such as a cytotoxin or a chemotherapeutic agent. Such conjugates may be referred to as immunoconjugates. As used herein, the term "immunoconjugate" refers to an antibody which is chemically or biologically linked to another moiety, such as a cytotoxin, a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a toxin, a peptide or protein or a therapeutic agent. The antibody may be linked to the cytotoxin,

radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, toxin, peptide or therapeutic agent at any location along the molecule so long as it is able to bind its target. Examples of immunoconjugates include antibody drug conjugates and antibody-toxin fusion proteins. In one embodiment, the agent may be a second different antibody to Vδ1. In certain
 5 embodiments, the antibody may be conjugated to an agent specific for a tumor cell or a virally infected cell. The type of therapeutic moiety that may be conjugated to the anti-Vδ1 antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. In one embodiment, the agent may be a second antibody, or fragment thereof, that binds to a molecule other than Vδ1.

10

Multi-specific antibodies

The antibodies of the present invention may be mono-specific or they may bind additional targets and are therefore bi-specific or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may be specific for more than one target
 15 polypeptide. Therefore, in one embodiment, the antibody or fragment thereof comprises a first binding specificity to Vδ1 and a second binding specificity for a second target epitope.

20

In various embodiments, the second target epitope is an epitope of a cancer antigen or a cancer-associated antigen. In various embodiments, the cancer antigen or cancer-associated antigen is one selected from AFP, AKAP-4, ALK, alphafetoprotein, Androgen receptor, B7H3, BAGE, BCA225, BCAA, Bcr-abl, beta-Catenin, beta-HCG, beta-human chorionic gonadotropin, BORIS, BTAA, CA 125, CA 15-3, CA 195, CA 19-9, CA 242, CA 27.29, CA 72-4, CA-50, CAM 17.1, CAM43, Carbonic anhydrase IX, carcinoembryonic antigen, CD22, CD33/IL3Ra, CD68/P1, CDK4, CEA, chondroitin sulfate proteoglycan 4 (CSPG4), c-Met, CO-
 25 029, CSPG4, Cyclin B1, cyclophilin C-associated protein, CYP1B1, E2A-PRL, EGFR, EGFRvIII, ELF2M, EpCAM, EphA2, EphrinB2, Epstein Barr virus antigens EBVA, ERG (TMPRSS2ETS fusion gene), ETV6-AML, FAP, FGF-5, Fos-related antigen 1, Fucosyl GM1, G250, Ga733/EpCAM, GAGE-1, GAGE-2, GD2, GD3, glioma-associated antigen, GloboH, Glycolipid F77, GM3, GP 100, GP 100 (Pmel 17), H4-RET, HER-2/neu, HER-2/Neu/ErbB-2,
 30 high-molecular-weight melanoma-associated antigen (HMW-MAA), HPV E6, HPV E7, hTERT, HTgp-175, human telomerase reverse transcriptase, Idiotype, IGF-I receptor, IGF-II, IGH-IGK, insulin growth factor (IGF)-I, intestinal carboxyl esterase, K-ras, LAGE-1a, LCK, lectin-reactive AFP, Legumain, LMP2, M344, MA-50, Mac-2 binding protein, MAD-CT-1, MAD-CT-2, MAGE, MAGE A1, MAGE A3, MAGE-1, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MART-1,
 35 MART-1/MelanA, M-CSF, melanoma-associated chondroitin sulfate proteoglycan (MCSP), Mesothelin, MG7-Ag, ML-IAP, MN-CA IX, MOV18, MUC1, Mum-1, hsp70-2, MYCN, MYL-RAR, NA17, NB/70K, neuron-glia antigen 2 (NG2), neutrophil elastase, nm-23H1, NuMa, NY-

- BR-1, NY-CO-1, NY-ESO, NY-ESO-1, NY-ESO-1, OY-TES1, p15, p16, p180erbB3, p185erbB2, p53, p53 mutant, Page4, PAX3, PAX5, PDGFR-beta, PLAC1, Polysialic Acid, prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen, prostatic acid phosphatase (PAP), Proteinase3 (PR1), PSA, PSCA, PSMA, RAGE-1, Ras, Ras-mutant, RCAS1, RGS5, RhoC, ROR1, RU1, RU2 (AS), SART3, SDCCAG16, sLe(a), Sperm protein 17, SSX2, STn, Survivin, TA-90, TAAL6, a TAG-72, telomerase, thyroglobulin, Tie 2, TIGIT, TLP, Tn, TPS, TRP-1, TRP-2, TRP-2, TSP-180, Tyrosinase, VEGFR2, VISTA, WT1, XAGE 1, 43-9F, 5T4, and 791Tgp72.
- 10 In various embodiments, the second target epitope is an epitope of a cluster of differentiation CD antigen. In various embodiments, the CD antigen is one selected from CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3, CD3d, CD3e, CD3g, CD4, CD5, CD6, CD7, CD8, CD8a, CD8b, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CD13, CD14, CD15, CD16, CD16a, CD16b, CD17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32A, CD32B, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60a, CD60b, CD60c, CD61, CD62E, CD62L, CD62P, CD63, CD64a, CD65, CD65s, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD77, CD79A, CD79B, CD80, CD81, CD82, CD83, CD84, CD85A, CD85B, CD85C, CD85D, CD85F, CD85G, CD85H, CD85I, CD85J, CD85K, CD85M, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120, CD120a, CD120b, CD121a, CD121b, CD122, CD123, CD124, CD125, CD126, CD127, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140A, CD140B, CD141, CD142, CD143, CD144, CDw145, CD146, CD147, CD148, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD156a, CD156b, CD156c, CD157, CD158, CD158A, CD158B1, CD158B2, CD158C, CD158D, CD158E1, CD158E2, CD158F1, CD158F2, CD158G, CD158H, CD158I, CD158J, CD158K, CD159a, CD159c, CD160, CD161, CD162, CD163, CD164, CD165, CD166, CD167a, CD167b, CD168, CD169, CD170, CD171, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179a, CD179b, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CD187, CD188, CD189, CD190, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CDw198, CDw199, CD200, CD201, CD202b, CD203c, CD204, CD205, CD206, CD207, CD208, CD209, CD210, CDw210a, CDw210b, CD211, CD212, CD213a1, CD213a2, CD214, CD215, CD216, CD217, CD218a, CD218b, CD219, CD220,

CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235a, CD235b, CD236, CD237, CD238, CD239, CD240CE, CD240D, CD241, CD242, CD243, CD244, CD245[17], CD246, CD247, CD248, CD249, CD250, CD251, CD252, CD253, CD254, CD255, CD256, CD257, CD258, CD259, CD260, CD261, CD262, CD263, CD264, CD265, CD266, CD267, CD268, CD269, CD270, CD271, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD280, CD281, CD282, CD283, CD284, CD285, CD286, CD287, CD288, CD289, CD290, CD291, CD292, CDw293, CD294, CD295, CD296, CD297, CD298, CD299, CD300A, CD300C, CD301, CD302, CD303, CD304, CD305, CD306, CD307, CD307a, CD307b, CD307c, CD307d, CD307e, CD308, CD309, CD310, CD311, CD312, CD313, CD314, CD315, CD316, CD317, CD318, CD319, CD320, CD321, CD322, CD323, CD324, CD325, CD326, CD327, CD328, CD329, CD330, CD331, CD332, CD333, CD334, CD335, CD336, CD337, CD338, CD339, CD340, CD344, CD349, CD351, CD352, CD353, CD354, CD355, CD357, CD358, CD360, CD361, CD362, CD363, CD364, CD365, CD366, CD367, CD368, CD369, CD370, and CD371.

15

While the mechanisms by which $\gamma\delta$ T-cells recognize antigens and distinguish between healthy and diseased cells are not fully understood (Ming Heng and Madalene Heng, *Antigen Recognition by $\gamma\delta$ T-Cells*. Madame Curie Bioscience Database [Internet], Austin (TX): Landes Bioscience; 2000-2013), the fact that $\gamma\delta$ T-cells are able to distinguish between healthy cells and diseased cells and exhibit remarkable diseased cell polycytotoxicity (see non-limiting example cell types in Table 1) this means that they can be leveraged to provide improved medicaments with improved therapeutic windows. Further, by leveraging such $\gamma\delta$ T-cell capabilities, there is provided an opportunity to treat disease while sparing healthy cells, by colocalizing $\gamma\delta$ T-cells with diseased cells even when a particular cancer antigen, inflammatory antigen, or pathogen antigen is either not known, or is also present on healthy cells, in a particular patient.

25

Table 1. Example cancer cells killed by polycytotoxic human V δ 1+ cells

Breast Cancer	
M-CSF7, T47D, MDA-MB-231	Mahvi et al (1993) Cancer Imm. Immunother. (1993) 37:181 – 186 Dutta I et al (2017). Front. Immunol. 8:776
Lung Cancer	
GLC1, N592	Ferrarini et al (1996) Jn of Nat. Cancer Inst., Volume 88 (7) pp 436–441
Pancreas Cancer	
panc89, QGP-1, PANC-1	Maeurer et al (1996) JEM 183 (4) 1681-1696 Kitayama 1993 Clin Exp Imm 93 (3) 442-7
Gastrointestinal Cancer	

HT29, HCT116, Y, SKCO1, Caco2, HCT116, Lovo, DLD-1, SW480	Wu, et al. (2015), Oncoimmunology, 4:3, e992749 Mikulak, et al (2019) JCI Insight. 4(24):e125884 Groh et al (1999) PNAS 96 (12) 6879-6884
Neuroblastoma	
LAN1, KELLY	Fisher et al (2014) Clin Cancer Res; 20(22); 5720–32
Melanoma	
A375	Cordova (2012) Plos ONE 7 (11) e49878
Ovarian Cancer	
OV-1063, SW626	Groh et al (1999) PNAS 96 (12) 6879-6884
Liver Cancer	
HepG2	Groh et al (1999) PNAS 96 (12) 6879-6884
Cervical Cancer	
HeLa	Groh et al (1999) PNAS 96 (12) 6879-6884
Prostate Cancer	
DV145, PC-3	Groh et al (1999) PNAS 96 (12) 6879-6884
Multiple Myeloma	
ARH77, U266	Knight et al (2012) Cytotherapy, 14:9, 1110- 1118,
AML	
KG-1, KASUMI-1, OCI-AML3, U937, HL60, MV4 11, AML193, HEL, THP-1	Lorenzo et al (2019) Canc Imm Res 7 (4)
CLL	
MEC-1	Almeida et al (2016) Clin Can Res 22 (23)

By way of one non-limiting example, recent studies with CD3xHER2 multi-specifics highlight the challenges of current or conventional approaches. Specifically, use of such conventional approaches can result in less favorable toxicity profiles. This is because like many other

5 tumour associated antigens (TAAs), the HER2 antigen is not only expressed in cancers such as breast cancer but is also expressed on healthy tissues such as heart cells. Hence use of CD3xHER2 medicaments which engage and co-localize all T-cells with HER2 positive cells can result in less favorable therapy windows or therapeutic indices. This is because such medicaments will engage all T-cells of which the vast majority in circulation will be $\alpha\beta$ T-cells

10 (CD4+ positive, CD8+ positive etc). And once $\alpha\beta$ T-cells are co-localized with HER2 positive cells, such conventional $\alpha\beta$ T-cells exhibit limited capabilities to spare HER2+ healthy cells and limited capabilities to kill only diseased HER2+ diseased cells. Consequently, and by way of this example, in cynomolgus studies whereby such CD3xHER2 bispecifics were administered, early euthanasia (even on the day of dosing) was required in some situations.

15 Further, during this example study (see Staflin et al. (2020) JCI Insight 5(7): e133757) it was concluded that retargeting T cells to kill HER2-expressing cells may induce adverse effects on

HER2-expressing tissues. It was noted that with exception of the liver, all affected or damaged tissues expressed HER2.

5 In a further non-limiting example, a second binding specificity may be to a tumour associated moiety also involved in controlling or regulating immune cell function. For example, the second specificity may be designed to target a so-called "checkpoint inhibitor" such as PD-L1 (CD274) or CD155. Once again, neither PDL-1 and CD155 are 100% disease specific. Both proteins can also be expressed on healthy cells. However, multi-specific antibodies designed to specifically co-localize V δ 1+ cells to either PD-L1 positive cells or CD155 positive cells may
10 result in the selective killing PD-L1 or CD155 positive diseased or cancerous cells. Further targeting diseased-associated checkpoint inhibitors present on diseased cells such as cancer cells will not only co-localize V δ 1+ cells to such tumours but may also confer additional favourable effects, for example by modulating or dampening PD-1/PD-L1 or TIGIT/CD155 signalling which otherwise may negatively regulates T cell-mediated immune responses to the
15 disease.

Hence instead of employing such conventional approaches, provided herein are multi-specific antibodies wherein at least one first binding specificity is able to bind V δ 1+ cells and at least one second binding specificity is able to bind targets present on diseased tissues and cells.
20 The use of such multi-specific antibodies in this way may thereby result in the co-localization of V δ 1+ cells to diseased cells expressing the second target. Further, and given such disease associated targets are not often 100% disease specific, this approach of targeting and co-localizing V δ 1+ effector cells specifically, may be more preferred over conventional approaches. This is because V δ 1+ effector cells may be capable of recognizing stress
25 patterns in diseased or infected cells and so able to selectively kill diseased cells whilst sparing healthy cells also expressing the same target.

In a further non-limiting example, a patient may have liver cancer, where no liver cancer specific antigen is known in the patient. In this instance, the second specificity of the multi-specific antibody can be to an epitope present on many or all liver cells, such as, for example, asialoglycoprotein receptor 1. This will then colocalize the $\gamma\delta$ T-cells to the liver, where the $\gamma\delta$ T-cells can kill the liver cancer cells, while sparing the healthy liver cells. By way of a third non-limiting example, in a patient that has lung cancer, where no lung cancer antigen is known in the patient, the second specificity of the multi-specific antibody can be to an epitope on a
30 normal lung cell, such as, for example, SP-1. This will colocalize the $\gamma\delta$ T-cells to the lung, where the $\gamma\delta$ T-cells can kill the lung cancer cells, while sparing the healthy lung cells. By way of a fourth non-limiting example, in a patient that has B cell lymphoma, where no B cell

lymphoma antigen is known in the patient, the second specificity of the multi-specific antibody can be to an epitope on normal B cells, such as, for example, CD19. This will colocalize the $\gamma\delta$ T-cells to B cells, where the $\gamma\delta$ T-cells can kill the lymphoma cells, while sparing healthy B cells. Cell-specific antigens, cell-associated antigens, tissue-specific antigens, and tissue-associated antigens are well known in the art and any such antigen can be targeted by the

5 second specificity of the multi-specific antibodies of the invention.

The second binding specificity may target an antigen on the same cell as V δ 1 or on a different cell of the same tissue type or of a different tissue type. In certain embodiments, the target

10 epitope may be on a different cell including a different T-cell, a B-cell, a tumour cell, an autoimmune tissue cell or a virally infected cell. Alternatively, the target epitope may be on the same cell.

The multi-specific antibodies, or fragments thereof, can be made in any format, so long as the

15 antibody, or fragment thereof, has multiple specificities. Examples of multi-specific antibody formats include, but are not limited to, CrossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, Knobs-in-holes (KIH), Knobs-in-holes (common light chain), Charge pair, Fab-arm exchange, SEEDbody, Triomab, LUZ-Y, Fcab, $\kappa\lambda$ -body, orthogonal Fab, DVD-IgG, IgG(H)-scFv, scFv(H)-IgG, IgG(L)-scFv, scFv(L)-IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG,

20 IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zybody, DVI-IgG(four-in-one), Nanobody, Nanoby-HAS, BiTE, Diabody, DART, TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, Triple Body, Morrison formats, Miniantibody, Minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')₂, F(ab)₂-scFv₂, scFv-KIH, Fab-scFv-Fc, Tetravalent HCAb, scDiabody-Fc, Diabody-Fc, Tandem scFv-Fc, Intrabody, Dock and Lock,

25 ImmTAC, HSAbody, scDiabody-HAS, Tandem scFv-Toxin, IgG-IgG, ov-X-Body, duobody, mab² and scFv1-PEG-scFv₂ (see Spiess et al. (2015) Molecular Immunology 67:95-106).

An antibody or fragment thereof as described herein may also be assessed by measuring its capacity for enhanced functionality in a multi-specific format such as a bispecific or trispecific

30 format. Surprisingly through such studies it is possible to identify yet further functional improvements in the performance of the antibodies or fragments thereof as described herein (see Example 20 and 21).

Various antibody-derived multi-specific formats have been described previously and are

35 typically built empirically from the component binding parts. Typically, once constructed, the performance of such multi-specific or multi-target binding formats as described herein may be measured in one or more of the aforementioned model systems (cell killing, cell proliferation,

healthy cell sparing/diseased cell specific models etc). Optionally they are also compared to said component parts and other comparator molecules.

Whilst not being limited by this approach, in general when constructing antibodies as multi-specific antibodies, the binding domain modules to each target (first, second, third etc) are optional built from scFv, Fab, Fab', F(ab')₂, Fv, variable domain (e.g. VH or VL), diabody, minibody or full length antibodies. For example, each said binding domain or module is created in one or more of the following non-limiting formats wherein binding domains comprising variable domains, and/or full length antibodies, and/or antibody fragments, are operatively linked in series to generate multi-specific antibodies.

Remarkably, multi-specific antibodies comprising at least one (first) binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR as described herein are further enhanced when said first binding domain is formatted with a multi-specific antibody format comprising at least one second binding domain against either tissue ("solid") and haemopoietic ("liquid") disease or cell-type associated targets.

Multi-specific antibodies - non-limiting examples:

To outline the applicability of the approach a series of non-limiting example multi-specific antibodies were constructed. These multi-specific antibodies comprised at least one (first) binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR and at least one (second) binding domain targeting a disease associated target:

A first example; V δ 1-EGFr multi-specific antibody:

For this example, one binding domain (to the first target) comprised intact antibody moieties; specifically, VH-CH1-CH2-CH3 and cognate VL-CL partners, whilst the second binding domain (to the second target) comprised an antibody fragment; specifically, a scFv format. The two binding modules were then fused with aid of a linker. The resulting bispecific format is sometimes termed a 'Morrison format'. In this instance a first binding domain targets the V δ 1 chain of a $\gamma\delta$ TCR and a second binding domain targets EGFr (see Example 20).

A second example; V δ 1-EGFr multi-specific antibody:

For this example, one binding domain (to the first target) comprised an antibody variable domain (specifically comprising a VH and cognate VL domain) whilst the second binding

domain (to the second target) comprises a binding domain within a heavy chain constant domain (CH1-CH2-CH3) (see also EP2546268 A1 Table 1 / EP3487885 A1). The resulting bispecific comprises a first binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR and a second binding domain targeting EGF receptor (see Example 20).

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A third example: V δ 1-CD19 multi-specific antibody:

For this example, one binding domain (to the first target) comprised intact antibody moieties specifically, VH-CH1-CH2-CH3 and cognate VL-CL partners, whilst the second binding domain
10 (to the second target) comprised an antibody fragment; specifically, a scFv format. The two binding modules were then fused with aid of a linker. For this example, the resulting bispecific comprised a first binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR and a second binding domain targeting CD19 (see Example 21).

15 Remarkably in all said examples comprising at least one (first) binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR at least one second domain targeting a second epitope enhanced functionality was observed versus the controls and component parts (see Examples 20 and 21 herein).

20 Collectively, these non-limiting examples highlight the flexibility of the antibodies or fragments thereof as described herein. These non-limiting examples outline that multi-specific antibody approach wherein antibodies or fragments thereof targeting the germline V δ 1 chain (amino acids 1-90 of SEQ ID NO:1) may be further enhanced by combining with second binding domains to form multi-specific antibodies. By way of non-limiting examples, multi-specific
25 antibodies are provided herein with enhanced functionality and which contain binding domains comprising intact antibodies (VH-CH1-CH2-CH3 and VL-CL), and/or variable domains (VH and cognate VL or VH-CH1 and cognate VL-CL), and/or antibody fragments (scFv).

In one embodiment multi-specific antibody binding domains which target V δ 1 chain of a $\gamma\delta$
30 TCR (the first target) may comprise (i) one or two or more antibody binding domains each comprising a heavy chain (VH-CH1-CH2-CH3) and a cognate light chain partner (VL-CL) and/or (ii) one or two or more antibody binding domains each comprising a heavy chain variable domain (VH, or VH-CH1) and a cognate light chain variable domain partner (VL, or VL-VC) and/or (iii) one or two or more antibody binding domains each comprising a CDR-
35 containing antibody fragment.

In one embodiment there is provided a multi-specific antibody comprising at least one first antibody-derived binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR and which is operatively linked to at least one second antibody binding domain targeting a second epitope. Optionally, said binding domains comprise at least one or more VH and cognate VL binding domain, or one or more VH-CH1-CH2-CH3 and cognate VL-CL binding domain, or one or more antibody fragment binding domains. Optionally, said second binding domain targets a second epitope associated with, or expressed on, the cell surface of a cell. Optionally, said second epitope is located on a cell surface polypeptide associated with a diseased cell or tumour cell or a virally infected cell or an autoimmune tissue cell. Optionally, said second epitope or epitopes are located on the disease and cell-type associated CD19 or EGFr antigens. Optionally, said multi-specific antibody comprising at least one first antibody-derived binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR is operatively linked to a second binding domain binding the EGF receptor and comprising one or more of the following heavy chain modifications in accordance with EU nomenclature; L358T and/or T359D and /or K360D and/or N361G and/or Q362P and/or N384T and/or G385Y and/or Q386G and/or D413S and/or K414Y and/or S415W and/or Q418Y and or Q419K.

Optionally, a multi-specific antibody comprising at least one first antibody-derived binding domain targeting the V δ 1 chain of a $\gamma\delta$ TCR is operatively linked to a second binding domain comprising SEQ ID NO:147 or SEQ ID NO: 148 or SEQ ID NO:149 or SEQ ID NO:157 or functionally equivalent binding variants thereof and which target either EGFr or CD19. Optionally, resulting multi-specific antibodies comprise SEQ ID NO: 140 or SEQ ID NO: 141 or SEQ ID NO:142 or SEQ ID NO: 144 or SEQ ID NO: 145 or SEQ ID NO: 146 or SEQ ID NO: 158 or SEQ ID NO: 159. Said entities are hereby included as non-limiting novel compositions to aid with understanding.

In one aspect of the invention multi-specific antibodies of the invention can be used in therapeutically effective amounts to treat a disease or disorder such to ameliorate at least one sign or symptom of a disease or disorder.

In one embodiment, there is provided a method of selecting or characterizing or comparing antibodies or fragment thereof as described herein which bind to the V δ 1 chain of a $\gamma\delta$ TCR in a multi-specific antibody format wherein said multi-specific antibody is applied to V δ 1+ cells in order to measure the conferred effect by said multi-specific entity V δ 1+ cells (e.g. upon said V δ 1+ phenotype and/or cytotoxicity and/or diseased-cell specificity and/or enhancement thereof).

Polynucleotides and expression vectors

In one aspect of the invention there is provided a polynucleotide encoding the anti-V δ 1 antibody or multi-specific antibody or fragment of the invention. In one embodiment, the polynucleotide comprises or consists of a sequence having at least 70%, such as at least 80%,
5 such as at least 90%, such as at least 95%, such as at least 99% sequence identity with SEQ ID NO: 99-110. In one embodiment, the expression vector comprises the VH region of SEQ ID NO: 99-110. In another embodiment, the expression vector comprises the VL region of SEQ ID NO: 99-110. In a further embodiment the polynucleotide comprises or consists of SEQ ID NO: 99-110. In a further aspect there is provided a cDNA comprising said polynucleotide.

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In one aspect of the invention there is provided a polynucleotide encoding the anti-V δ 1 antibody or fragment of the invention. In one embodiment, the polynucleotide comprises or consists of a sequence having at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with SEQ ID NO: 99-101 or 105-108.
15 In one embodiment, the expression vector comprises the VH region of SEQ ID NO: 99-101 or 105-108. In another embodiment, the expression vector comprises the VL region of SEQ ID NO: 99-101 or 105-108. In a further embodiment the polynucleotide comprises or consists of SEQ ID NO: 99-101 or 105-108. In a further aspect there is provided a cDNA comprising said polynucleotide.

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In one embodiment, the polynucleotide comprises or consists of a sequence having at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with SEQ ID NO: 99-101. In one embodiment, the expression vector comprises the VH region of SEQ ID NO: 99-101. In another embodiment, the expression
25 vector comprises the VL region of SEQ ID NO: 99-101. In a further embodiment the polynucleotide comprises or consists of SEQ ID NO: 99-101. In a further aspect there is provided a cDNA comprising said polynucleotide.

In one aspect of the invention there is provided a polynucleotide comprising or consisting of a
30 sequence having at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with any one of the portions of SEQ ID NO: 99-110 which encodes CDR1, CDR2 and/or CDR3 of the encoded immunoglobulin chain variable domain. In one embodiment, the polynucleotide comprises or consists of a sequence having at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at
35 least 99% sequence identity with any one of the portions of SEQ ID NO: 99-101 or 105-108 which encodes CDR1, CDR2 and/or CDR3 of the encoded immunoglobulin chain variable domain. In one embodiment, the polynucleotide comprises or consists of a sequence having

at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with any one of the portions of SEQ ID NO: 99-101 which encodes CDR1, CDR2 and/or CDR3 of the encoded immunoglobulin chain variable domain.

- 5 In one aspect of the invention there is provided a polynucleotide comprising or consisting of a sequence having at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with any one of the portions of SEQ ID NO: 99-110 which encodes FR1, FR2, FR3 and/or FR4 of the encoded immunoglobulin chain variable domain. In one embodiment, the polynucleotide comprises or consists of a sequence having
- 10 at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with any one of the portions of SEQ ID NO: 99-101 or 105-108 which encodes FR1, FR2, FR3 and/or FR4 of the encoded immunoglobulin chain variable domain. In one embodiment, the polynucleotide comprises or consists of a sequence having
- 15 at least 70%, such as at least 80%, such as at least 90%, such as at least 95%, such as at least 99% sequence identity with any one of the portions of SEQ ID NO: 99-101 which encodes FR1, FR2, FR3 and/or FR4 of the encoded immunoglobulin chain variable domain.

The polynucleotides and expression vectors of the invention may also be described in reference to the amino acid sequence encoded. Therefore, in one embodiment, the

20 polynucleotide comprises or consists of a sequence encoding the amino acid sequence of any one of SEQ ID NOs: 62 to 85. In one embodiment, the expression vector comprises a sequence encoding the amino acid sequence of any one of SEQ ID NOs: 62 to 73. In another embodiment, the expression vector comprises a sequence encoding the amino acid sequence of any one of SEQ ID NOs: 74 to 85.

25 To express the antibodies, or fragments thereof, polynucleotides encoding partial or full-length light and heavy chains, as described herein, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. Therefore, in one aspect of the invention there is provided an expression vector comprising the

30 polynucleotide sequence as defined herein. In one embodiment, the expression vector comprises the VH region of SEQ ID NO: 99-110, such as SEQ ID NO: 99, 100, 101, 105, 106, 107 or 108. In another embodiment, the expression vector comprises the VL region of SEQ ID NO: 99-110, such as SEQ ID NO: 99, 100, 101, 105, 106, 107 or 108.

35 It will be understood that the nucleotide sequences described herein comprise additional sequences encoding amino acid residues to aid with translation, purification and detection, however alternative sequences may be used depending upon the expression system used.

For example, the initial (5'-end) nine nucleotides of SEQ ID NOs: 99-110 and the final (3'-end) 36 nucleotides of SEQ ID NOs: 99-100, 102-103, 105-110, or the final (3'-end) 39 nucleotides of SEQ ID NOs: 101 and 104 are optional sequences. These optional sequences can be removed, modified or substituted if alternate design, translation, purification or detection strategies are adopted.

Mutations can be made to the DNA or cDNA that encode polypeptides which are silent as to the amino acid sequence of the polypeptide, but which provide preferred codons for translation in a particular host. The preferred codons for translation of a nucleic acid in, e.g. *E. coli* and *S. cerevisiae*, as well as mammalian, specifically human, are known.

Mutation of polypeptides can be achieved for example by substitutions, additions or deletions to a nucleic acid encoding the polypeptide. The substitutions, additions or deletions to a nucleic acid encoding the polypeptide can be introduced by many methods, including for example error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, artificial gene synthesis, Gene Site Saturation Mutagenesis (GSSM), synthetic ligation reassembly (SLR) or a combination of these methods. The modifications, additions or deletions to a nucleic acid can also be introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, or a combination thereof.

In particular, artificial gene synthesis may be used. A gene encoding a polypeptide of the invention can be synthetically produced by, for example, solid-phase DNA synthesis. Entire genes may be synthesized *de novo*, without the need for precursor template DNA. To obtain the desired oligonucleotide, the building blocks are sequentially coupled to the growing oligonucleotide chain in the order required by the sequence of the product. Upon the completion of the chain assembly, the product is released from the solid phase to solution, deprotected, and collected. Products can be isolated by high-performance liquid chromatography (HPLC) to obtain the desired oligonucleotides in high purity.

Expression vectors include, for example, plasmids, retroviruses, cosmids, yeast artificial chromosomes (YACs) and Epstein-Barr virus (EBV) derived episomes. The polynucleotide is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the polynucleotide. Expression and/or control sequences can include promoters, enhancers, transcription terminators, a start codon (*i.e.* ATG) 5' to the coding sequence, splicing signals for introns and stop codons. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. SEQ ID NO: 99-110 comprise the nucleotide sequences encoding single chain variable fragments of the invention, comprising a VH region and a VL region joined by a synthetic linker (encoding SEQ ID NO: 98). It will be understood that polynucleotides or expression vectors of the invention may comprise the VH region, the VL region or both (optionally including the linker). Therefore, polynucleotides encoding the VH and VL regions can be inserted into separate vectors, alternatively sequences encoding both regions are inserted into the same expression vector. The polynucleotide(s) are inserted into the expression vector by standard methods (*e.g.* ligation of complementary restriction sites on the polynucleotide and vector, or blunt end ligation if no restriction sites are present).

A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed, as described herein. The expression vector can also encode a signal peptide that facilitates secretion of the antibody (or fragment thereof) from a host cell. The polynucleotide may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.* a signal peptide from a non-immunoglobulin protein).

In one aspect of the invention there is provided a cell (*e.g.* a host cell) comprising the polynucleotide or expression vector as defined herein. It will be understood that the cell may comprise a first vector encoding the light chain of the antibody or fragment thereof, and a second vector encoding the heavy chain of the antibody or fragment thereof. Alternatively, the heavy and light chains both encoded on the same expression vector introduced into the cell.

In one embodiment, the polynucleotide or expression vector encodes a membrane anchor or transmembrane domain fused to the antibody or fragment thereof, wherein the antibody or fragment thereof is presented on an extracellular surface of the cell.

Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, biolistic injection and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, inter alia, Chinese hamster ovary (CHO) cells, NSO, SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g. Hep G2), A549 cells, 3T3 cells, and a number of other cell lines. Mammalian host cells include human, mouse, rat, dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells, bacterial cells, plant cells and fungal cells. Antigen-binding fragments of antibodies such as the scFv and Fv fragments can be isolated and expressed in *E. coli* using methods known in the art.

The antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Antibodies (or fragments) of the invention can be obtained and manipulated using the techniques disclosed for example in Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (2012) 4th Edition Cold Spring Harbour Laboratory Press.

Monoclonal antibodies can be produced using hybridoma technology, by fusing a specific antibody-producing B cell with a myeloma (B cell cancer) cell that is selected for its ability to grow in tissue culture and for an absence of antibody chain synthesis.

A monoclonal antibody directed against a determined antigen can, for example, be obtained by:

- a) immortalizing lymphocytes obtained from the peripheral blood of an animal previously immunized with a determined antigen, with an immortal cell and preferably with myeloma cells, in order to form a hybridoma,
- b) culturing the immortalized cells (hybridoma) formed and recovering the cells producing the antibodies having the desired specificity.

Alternatively, the use of a hybridoma cell is not required. Antibodies capable of binding to the target antigens as described herein may be isolated from a suitable antibody library via routine practice, for example, using the phage display, yeast display, ribosomal display, or mammalian display technology known in the art. Accordingly, monoclonal antibodies can be obtained, for example, by a process comprising the steps of:

- a) cloning into vectors, especially into phages and more particularly filamentous bacteriophages, DNA or cDNA sequences obtained from lymphocytes especially peripheral blood lymphocytes of an animal (suitably previously immunized with determined antigens),
- b) transforming prokaryotic cells with the above vectors in conditions allowing the production of the antibodies,
- c) selecting the antibodies by subjecting them to antigen-affinity selection,
- d) recovering the antibodies having the desired specificity.

Optionally, isolated polynucleotide encoding antibodies or fragment thereof as described herein and which bind to the V δ 1 chain of a $\gamma\delta$ can also be readily manufactured to make sufficient quantities to be employed as a medicaments to ameliorate the signs or symptoms of disease. When employed as a medicament in this manner, typically the polynucleotides of interest are first operatively linked to an expression vector or expression cassette designed to express said antibodies or fragment thereof in a subject or patient. Such expression cassettes and methods of delivery of polynucleotides or what are sometime termed 'nucleic-based' medicaments are well known in the art. For recent review see Hollevoet and Declerck (2017) J. Transl. Med. 15(1): 131.

Pharmaceutical compositions

According to a further aspect of the invention, there is provided a composition comprising the antibody or fragment thereof as defined herein. In such embodiments, the composition may comprise the antibody, optionally in combination with other excipients. Also included are compositions comprising one or more additional active agents (e.g. active agents suitable for treating the diseases mentioned herein).

According to a further aspect of the invention, there is provided a pharmaceutical composition comprising the antibody or fragment thereof as defined herein, together with a pharmaceutically acceptable diluent or carrier. The antibodies of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject.

- 5 Typically, the pharmaceutical composition comprises an antibody of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, salts, 10 phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance 15 the shelf life or effectiveness of the antibody or fragment thereof.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g. injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories.

- 20 The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions.

The preferred mode of administration is parenteral (e.g. intravenous, subcutaneous, intraperitoneal, intramuscular, intrathecal). In a preferred embodiment, the antibody is 25 administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

- Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, 30 dispersion, liposome, or other ordered structure suitable to high drug concentration.

- It is within the scope of the invention to use the pharmaceutical composition of the invention in therapeutic methods for the treatment of diseases as described herein as an adjunct to, or in conjunction with, other established therapies normally used in the treatment of such 35 diseases.

In a further aspect of the invention, the antibody, composition or pharmaceutical composition is administered sequentially, simultaneously or separately with at least one active agent.

Treatment methods

- 5 According to a further aspect of the invention, there is provided an isolated anti-V δ 1 antibody or fragment thereof as defined herein for use as a medicament. References herein to an antibody or fragment thereof "for use" as a medicament or in therapy are limited to administration of the antibody or fragment thereof to a subject. Such uses do not include administration of the antibody or fragment thereof to a cell culture (i.e. *in vitro* or *ex vivo*)
10 wherein said cell culture or derived cell therapy product is used as a therapeutic.

- In one embodiment, the anti-V δ 1 antibody or fragment thereof is for use in the treatment of cancer, an infectious disease or an inflammatory disease. In one embodiment, the invention is a method of treating a disease or disorder in a subject in need thereof, comprising the step
15 of administering an anti-V δ 1 antibody or fragment thereof to the subject. In various embodiments, the disease or disorder is cancer, an infectious disease or an inflammatory disease. In one embodiment, the anti-V δ 1 antibody or fragment thereof is for use in the treatment of cancer, an infectious disease or an inflammatory disease, leads to the death of diseased cells while sparing healthy cells. In a further embodiment, the antibody or fragment
20 thereof is for use in the treatment of cancer.

- In one embodiment, the antibody or fragment thereof is for use in the treatment of cancer, an infectious disease or an inflammatory disease. In a further embodiment, the antibody or fragment thereof is for use in the treatment of cancer.
25

- According to a further aspect of the invention, there is provided the pharmaceutical composition as defined herein for use as a medicament. In one embodiment, the pharmaceutical composition is for use in the treatment of cancer, an infectious disease or an inflammatory disease. In a further embodiment, the pharmaceutical composition is for use in
30 the treatment of cancer.

- According to a further aspect of the invention, there is provided a method of modulating an immune response in a subject in need thereof comprising administering a therapeutically effective amount of the isolated anti-V δ 1 antibody or fragment thereof as defined herein. In
35 various embodiments, modulating an immune response in a subject comprises binding or targeting $\gamma\delta$ T cells, activating $\gamma\delta$ T cells, causing or increasing proliferation of $\gamma\delta$ T cells, causing or increasing expansion of $\gamma\delta$ T cells, causing or increasing $\gamma\delta$ T cell degranulation,

causing or increasing $\gamma\delta$ T cell killing activity, causing or increasing $\gamma\delta$ T cell killing activity while sparing healthy cells, causing or increasing $\gamma\delta$ T cytotoxicity, causing or increasing $\gamma\delta$ T cytotoxicity while sparing healthy cells, causing or increasing $\gamma\delta$ T cell mobilization, increasing survival of $\gamma\delta$ T cells, or increasing resistance to exhaustion of $\gamma\delta$ T cells.

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According to a further aspect of the invention, there is provided method of treating a cancer, an infectious disease or an inflammatory disease in a subject in need thereof, comprising administering a therapeutically effective amount of the isolated anti-V δ 1 antibody or fragment thereof as defined herein. Alternatively, a therapeutically effective amount of the pharmaceutical composition is administered.

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According to further aspects of the invention, there is provided the use of an antibody or fragment thereof as defined herein for the manufacture of a medicament, for example in the treatment of cancer, an infectious disease or an inflammatory disease.

15

In one embodiment, the antibody or fragment thereof is administered to a subject, wherein the subject has cancer, an infectious disease or an inflammatory disease.

According to a further aspect of the invention, there is provided the pharmaceutical composition as defined herein for use as a medicament. In one embodiment, the pharmaceutical composition is administered to a subject, wherein the subject has cancer, an infectious disease or an inflammatory disease.

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According to a further aspect of the invention, there is provided a method of administering a therapeutically effective amount of the isolated anti-V δ 1 antibody or fragment thereof as defined herein to a subject, wherein the subject has cancer, an infectious disease or an inflammatory disease. Alternatively, a therapeutically effective amount of the pharmaceutical composition is administered.

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According to further aspects of the invention, there is provided the use of an antibody or fragment thereof as defined herein for the manufacture of a medicament, for example for the administration to a subject, wherein the subject has cancer, an infectious disease or an inflammatory disease.

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In various embodiments, the cancer that can be treated by the disclosed methods and compositions include, but are not limited to acute lymphoblastic, acute myeloid leukemia, adrenocortical carcinoma, appendix cancer, basal cell carcinoma, bile duct cancer, bladder

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cancer, bone cancer, osteosarcoma and malignant fibrous histiocytoma, brain stem glioma, brain tumor, brain tumor, brain stem glioma, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, craniopharyngioma, ependymoblastoma, ependymoma,

5 medulloblastoma, medulloepithelioma, pineal parenchymal tumors of intermediate differentiation, supratentorial primitive neuroectodermal tumors and pineoblastoma, visual pathway and hypothalamic glioma, brain and spinal cord tumors, breast cancer, bronchial tumors, Burkitt lymphoma, carcinoid tumor, gastrointestinal carcinoid tumor, central nervous system atypical teratoid/rhabdoid tumor, central nervous system embryonal tumors, central

10 nervous system lymphoma, cerebellar astrocytoma cerebral astrocytoma/malignant glioma, cervical cancer, chordoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, esophageal cancer, Ewing family of tumors, extragonadal germ cell tumor, extrahepatic bile duct cancer, intraocular melanoma, retinoblastoma, gallbladder

15 cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (gist), germ cell tumor, gestational trophoblastic tumor, glioma, glioma brain stem, glioma cerebral astrocytoma, glioma visual pathway and hypothalamic, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Langerhans cell histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, intraocular

20 melanoma, islet cell tumors, kidney (renal cell) cancer, Langerhans cell histiocytosis, laryngeal cancer, acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, hairy cell leukemia, lip and oral cavity cancer, liver cancer, non-small cell lung cancer, small cell lung cancer, aids-related lymphoma, Burkitt lymphoma, cutaneous T-cell lymphoma, non-Hodgkin lymphoma, primary central nervous system

25 lymphoma, Waldenstrom macroglobulinemia, malignant fibrous histiocytoma of bone and osteosarcoma, medulloblastoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm, mycosis, fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous

30 leukemia, myeloid leukemia, myeloid leukemia acute, multiple myeloma, myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, oral cancer, oral cavity cancer, oropharyngeal cancer, osteosarcoma and malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer,

35 pancreatic cancer, papillomatosis, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal parenchymal tumors of intermediate differentiation, pineoblastoma and supratentorial primitive neuroectodermal tumors, pituitary tumor, plasma

- celt neoplasm/multiple myeloma, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell (kidney) cancer, renal pelvis and ureter, respiratory tract carcinoma involving the nut gene on chromosome 15, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcoma, Ewing family of tumors, Kaposi
- 5 sarcoma, soft tissue sarcoma, uterine sarcoma, Sezary syndrome, skin cancer (nonmelanoma), skin cancer (melanoma), Merkel cell skin carcinoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach (gastric) cancer, supratentorial primitive neuroectodermal tumors, T-cell lymphoma, testicular cancer, throat cancer, thymoma and thymic carcinoma, thyroid cancer, transitional
- 10 cell cancer of the renal pelvis and ureter, gestational trophoblastic tumor, urethral cancer, uterine cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, and Wilms tumor. In various embodiments, the cancer that can be treated by the disclosed methods and compositions is treated while healthy cells are spared,
- 15 In various embodiments, the inflammatory diseases that can be treated by the disclosed methods and compositions include, but are not limited to Achalasia, Acute disseminated encephalomyelitis (ADEM), Acute motor axonal neuropathy, Acute respiratory distress syndrome (ARDS), Addison's disease, Adiposis dolorosa, Adult Still's disease, Adult-onset Still's disease, Agammaglobulinemia, Alopecia Areata, Amyloidosis, Amyotrophic lateral
- 20 sclerosis, Ankylosing spondylitis, Anti-GBM/Anti-TBM nephritis, Anti-N-Methyl-D-Aspartate (Anti-NMDA) Receptor Encephalitis, Antiphospholipid syndrome, Antiphospholipid syndrome (APS, APLS), Antisynthetase syndrome, Anti-tubular basement membrane nephritis, Aplastic anemia, Atopic allergy, Atopic dermatitis, Autoimmune angioedema, Autoimmune comorbidities, Autoimmune dysautonomia, Autoimmune encephalomyelitis, Autoimmune
- 25 enteropathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease (AIED), Autoimmune lymphoproliferative syndrome, Autoimmune myocarditis, Autoimmune neutropenia, Autoimmune oophoritis, Autoimmune orchitis, Autoimmune pancreatitis (AIP), Autoimmune peripheral neuropathy, Autoimmune polyendocrine syndrome (APS) type 1, Autoimmune polyendocrine syndrome (APS) type 2, Autoimmune polyendocrine
- 30 syndrome (APS) type 3, Autoimmune retinopathy, Autoimmune thrombocytopenic purpura, Autoimmune thyroiditis, Autoimmune urticaria, Autoimmune uveitis, Autoimmune vasculitis, Axonal & neuronal neuropathy (AMAN), Balo concentric sclerosis, Baló disease, Behcet's disease, Benign mucosal pemphigoid, Bickerstaff's encephalitis, Blau syndrome, Bullous pemphigoid, Castleman disease (CD), Celiac disease, Chagas disease, Chronic fatigue
- 35 syndrome, Chronic inflammatory demyelinating polyneuropathy (CIDP), Chronic obstructive pulmonary disease, Chronic recurrent multifocal osteomyelitis (CRMO), Churg-Strauss Syndrome (CSS) or Eosinophilic Granulomatosis (EGPA), Cicatricial pemphigoid, Cogan

syndrome, Cold agglutinin disease, Complement component 2 deficiency, Complex regional
 pain syndrome, Congenital heart block, Connective tissue, systemic, and multi-organ, Contact
 dermatitis, Coxsackie myocarditis, CREST syndrome, Crohn's disease, Cushing's syndrome,
 Cutaneous leukocytoclastic angiitis, Dego's disease, Dermatitis herpetiformis,
 5 Dermatomyositis, Devic's Disease (neuromyelitis optica), Diabetes mellitus type 1, Digestive
 system, Discoid lupus, Dressler's syndrome, Drug-induced lupus, Eczema, Endometriosis,
 Entesitis-related arthritis, Eosinophilic esophagitis (EoE), Eosinophilic fasciitis, Eosinophilic
 gastroenteritis, Eosinophilic granulomatosis with polyangiitis (EGPA), Eosinophilic
 pneumonia, Epidermolysis bullosa acquisita, Erythema nodosum, Erythroblastosis fetalis,
 10 Esophageal achalasia, Essential mixed cryoglobulinemia, Evans syndrome, Exocrine, Feltz
 syndrome, Fibrodysplasia ossificans progressiva, Fibromyalgia, Fibrosing alveolitis, Gastritis,
 Gastrointestinal pemphigoid, Giant cell arteritis (temporal arteritis), Giant cell myocarditis,
 Glomerulonephritis, Goodpasture's syndrome, Granulomatosis with Polyangiitis, Graves'
 ophthalmopathy, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis,
 15 Hashimoto's encephalopathy, Hemolytic anemia, Henoch-Schönlein purpura (HSP), Herpes
 gestationis or pemphigoid gestationis (PG), Hidradenitis Suppurativa (HS) (Acne Inversa),
 Hypogammaglobulinemia, Idiopathic giant-cell myocarditis, Idiopathic inflammatory
 demyelinating diseases, Idiopathic pulmonary fibrosis, IgA Nephropathy, IgA vasculitis (IgAV),
 IgG4-related disease, IgG4-related sclerosing disease, Immune thrombocytopenic purpura
 20 (ITP), Inclusion body myositis (IBM), Inflammatory Bowel Disease, Intermediate uveitis,
 Interstitial cystitis (IC), Interstitial lung disease, IPEX syndrome, Juvenile arthritis, Juvenile
 diabetes (Type 1 diabetes), Juvenile myositis (JM), Kawasaki disease, Lambert-Eaton
 syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Ligneous
 conjunctivitis, Linear IgA disease (LAD), Lupus, Lupus nephritis, Lupus vasculitis, Lyme
 25 disease chronic, Majeeb syndrome, Meniere's disease, Microscopic polyangiitis (MPA), Mixed
 connective tissue disease (MCTD), Mooren's ulcer, Morphea, Mucha-Habermann disease,
 Multifocal Motor Neuropathy (MMN) or MMNCB, Multiple sclerosis, Myasthenia gravis,
 Myocarditis, Myositis, Narcolepsy, Neonatal Lupus, Nervous system, Neuromyelitis optica,
 Neuromyotonia, Neutropenia, Ocular cicatricial pemphigoid, Opsoclonus myoclonus
 30 syndrome, Optic neuritis, Ord's thyroiditis, Oshtoran syndrome, Palindromic rheumatism (PR),
 Paraneoplastic cerebellar degeneration (PCD), Paroxysmal nocturnal hemoglobinuria (PNH),
 Parry-Romberg syndrome, Parsonage-Turner syndrome, Pars Planitis (peripheral uveitis),
 Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS),
 Pelvic Inflammatory Disease (PID), Pemphigus, Pemphigus vulgaris, Peripheral neuropathy,
 35 Perivenous encephalomyelitis, Pernicious anemia (PA), Pityriasis lichenoides et varioliformis
 acuta, POEMS syndrome, Polyarteritis nodosa, Polyglandular syndromes type I, II, III,
 Polymyalgia rheumatica, Polymyositis, Postmyocardial infarction syndrome,

Postpericardiotomy syndrome, Primary biliary cholangitis (PBC), Primary biliary cirrhosis, Primary immunodeficiency, Primary sclerosing cholangitis, Progesterone dermatitis, Progressive inflammatory neuropathy, Psoriasis, Psoriatic arthritis, Pure red cell aplasia (PRCA), Pyoderma gangrenosum, Rasmussen's encephalitis, Raynaud's phenomenon,

5 Reactive Arthritis, Reflex sympathetic dystrophy, Relapsing polychondritis, Restless legs syndrome (RLS), Retroperitoneal fibrosis, Rheumatic fever, Rheumatoid arthritis, Rheumatoid vasculitis, Sarcoidosis, Schizophrenia, Schmidt syndrome, Schnitzler syndrome, Scleritis, Scleroderma, Serum sickness, Sjögren's Syndrome, Sperm & testicular autoimmunity, Spondyloarthropathy, Stiff person syndrome (SPS), Subacute bacterial endocarditis (SBE),

10 Susac's Syndrome, Sweet's syndrome, Sydenham's chorea, Sympathetic ophthalmia (SO), Systemic lupus erythematosus (SLE), Takayasu's arteritis, Temporal arteritis/Giant cell arteritis, Thrombocytopenia, Thrombocytopenic purpura (TTP), Thyroid gland, Tolosa-Hunt syndrome (THS), Transverse myelitis, Type 1 diabetes, Ulcerative colitis (UC), Undifferentiated connective tissue disease (UCTD), Undifferentiated spondyloarthropathy, Urticarial vasculitis,

15 Urticaria, Uveitis, Vasculitis, Vitiligo, and Vogt-Koyanagi-Harada Disease. In various embodiments, the inflammatory disease that can be treated by the disclosed methods and compositions is treated, while healthy cells are spared.

In various embodiments, the infectious disease that can be treated by the disclosed methods

20 and compositions include, but are not limited to Acinetobacter infection, Actinomycosis, Acute Flaccid Myelitis (AFM), African sleeping sickness (African trypanosomiasis), AIDS (acquired immunodeficiency syndrome), Ameba infection, Amebiasis, Anaplasma phagocytophilum infection, Anaplasmosis, Angiostrongyliasis, Anisakiasis, Anthrax, Arboviral diseases, neuroinvasive and non-neuroinvasive, Arcanobacterium haemolyticum infection, Argentine

25 hemorrhagic fever, Ascariasis, Aspergillosis, Astrovirus infection, Avian Influenza, Babesiosis, Bacillus cereus infection, Bacterial infection, Bacterial meningitis, Bacterial pneumonia, Bacterial vaginosis, Bacteroides infection, Balantidiasis, Bartonellosis, Baylisascaris infection, BK virus infection, Black piedra, Blastocystosis, Bolivian hemorrhagic fever, Botulism, Botulism (foodborne), Botulism (infant), Botulism (other), Botulism (wound), Brazilian

30 hemorrhagic fever, Brucellosis, Bubonic plague, Burkholderia infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), California serogroup virus diseases, Campylobacter, Campylobacteriosis, Candida auris, clinical, Candidiasis (Moniliasis; Thrush), Capillariasis, Carbapenemase Producing Carbapenem-Resistant Enterobacteriaceae (CP-CRE), Carbapenem-resistant Infection (CRE/CRPA), Carrion's disease, Cat-scratch disease,

35 Cellulitis, Chagas disease (trypanosomiasis), Chancroid, Chickenpox, Chikungunya Virus Infection (Chikungunya), Chlamydia, Chlamydia trachomatis, Chlamydomonas pneumoniae infection, Cholera, Chromoblastomycosis, Chytridiomycosis, Ciguatera, Clonorchiasis,

Clostridium difficile colitis, Clostridium Difficile Infection, Clostridium perfringens, Coccidioidomycosis fungal infection (Valley fever), Colorado tick fever (CTF), Common cold (Acute viral rhinopharyngitis; Acute coryza), Congenital syphilis, Conjunctivitis, COVID-19 (Coronavirus Disease 2019), CP-CRE, Enterobacter spp., CP-CRE, Escherichia coli (E. coli),
 5 CP-CRE, Klebsiella spp., Creutzfeldt-Jacob Disease, transmissible spongiform encephalopathy (CJD), Creutzfeldt-Jakob disease (CJD), Crimean-Congo hemorrhagic fever (CCHF), Crusted Scabies, Cryptococcosis, Cryptosporidiosis (Crypto), Cutaneous larva migrans (CLM), Cyclospora, Cyclosporiasis, Cysticercosis, Cytomegalovirus infection, Dengue virus infections, Dengue, 1,2,3,4 (Dengue Fever), Dengue-like illness, Desmodermis
 10 infection, Diarrheal Illness, Dientamoebiasis, Diphtheria, Diphyllbothriasis, Dracunculiasis, E. coli, E. coli infection, Shiga toxin-producing (STEC), Eastern equine encephalitis virus disease, Ebola Hemorrhagic Fever (Ebola), Echinococcosis, Ehrlichia chaffeensis infection, Ehrlichia ewingii infection, Ehrlichiosis, Anaplasmosis, Encephalitis, Arboviral or parainfectious, Enterobiasis (Pinworm infection), Enterococcus infection,, Enterovirus
 15 Infection, D68 (EV-D68), Enterovirus Infection, Non-Polio (Non-Polio Enterovirus), Epidemic typhus, Epstein-Barr virus infectious mononucleosis (Mono), Erythema infectiosum (Fifth disease), Exanthem subitum (Sixth disease), Fascioliasis, Fasciolopsiasis, Fatal familial insomnia (FFI), Fifth Disease, Filariasis, Flu (Seasonal), Food Poisoning, Food poisoning by Clostridium perfringens, Free-living amebic infection, Fungal infection, Fusobacterium
 20 infection, Gas gangrene (Clostridial myonecrosis), Genital Herpes, Genital Warts, Geotrichosis, German Measles, Gerstmann-Sträussler-Scheinker syndrome (GSS), Giardiasis, Glanders, Gnathostomiasis, Gonorrhea, Granuloma inguinale, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group A Streptococcus, Group B streptococcal infection, Guanarito virus, Haemophilus Influenza disease, Type B (Hib or H-
 25 flu), Haemophilus influenzae infection, Hand, foot and mouth disease (HFMD), Hansen's Disease, Hantavirus infection, Hantavirus Pulmonary Syndrome (HPS), Heartland virus disease, Helicobacter pylori infection, Hemolytic Uremic Syndrome (HUS), Hemorrhagic fever with renal syndrome (HFRS), Hendra virus infection, Hepatitis A (Hep A), Hepatitis B (Hep B), Hepatitis C (Hep C), Hepatitis D (Hep D), Hepatitis E (Hep E), Herpes, Herpes B Virus, Herpes
 30 simplex, Herpes Zoster, zoster VZV (Shingles), Hib Disease, Histoplasmosis infection (Histoplasmosis), Hookworm infection, HPV (Human Papillomavirus), Human bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis (HGA), Human Immunodeficiency Virus/AIDS (HIV/AIDS), Human metapneumovirus infection, Human monocytic ehrlichiosis, Human papillomavirus (HPV) infection, Human parainfluenza virus
 35 infection, Hymenolepiasis, Impetigo, Influenza (flu), Influenza (Seasonal), Invasive pneumococcal disease, Isosporiasis, Junin virus,, Kawasaki Syndrome, Keratitis, Kingella kingae infection, Kuru, Lassa fever, Lassa virus, Legionellosis (Legionnaires' disease),

- Leishmaniasis, Leprosy (Hansens Disease), Leptospirosis, Listeriosis (Listeria), Lujo virus, Lyme disease, Lymphatic filariasis (Elephantiasis), Lymphocytic Choriomeningitis (LCMV), Lymphogranuloma venereum infection (LGV), Machupo virus, Malaria, Marburg virus infection, Measles, Melioidosis (Whitmore's disease), Meningitis, Meningitis – Bacterial,
- 5 Meningitis – Viral, Meningococcal disease, Metagonimiasis, Microsporidiosis, Middle East respiratory syndrome (MERS), Molluscum contagiosum (MC), Monkeypox, Mononucleosis, Mosquito-borne Illness, MRSA, Mumps,, Murine typhus (Endemic typhus), Mycetoma, Mycoplasma genitalium infection, Mycoplasma pneumonia, Myiasis, Neisseria meningitidis, Neonatal conjunctivitis (Ophthalmia neonatorum), Nipah virus infection, Nocardiosis,
- 10 Norovirus, Onchocerciasis (River blindness), Opisthorchiasis, Orf Virus (Sore Mouth), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Paralytic Shellfish Poisoning (Paralytic Shellfish Poisoning, Ciguatera), Pasteurellosis, PEP, Parasitic infection, Pertussis (whooping cough), Pink Eye, Pneumococcal Disease, Pneumococcal infection, Pneumocystis pneumonia (PCP), Pneumonia, Pneumonic Plague, Poliomyelitis (Polio),
- 15 Poliomyelitis, paralytic, Poliovirus infection, Pontiac fever, Powassan virus disease, Prevotella infection, Primary amoebic meningoencephalitis (PAM), Progressive multifocal leukoencephalopathy, Protozoan infection, Psittacosis (Parrot Fever), Pustular Rash diseases (Small pox, monkeypox, cowpox), Rabies, Raccoon Roundworm, Rat Bite Fever, Recreational Water Illnesses, Relapsing fever, Respiratory syncytial virus infection, Reye's Syndrome,
- 20 Rhinosporidiosis, Rhinovirus infection, Rickettsial infection, Rickettsiosis (Rocky Mountain Spotted Fever), Rift Valley fever (RVF), Ringworm, Rotavirus infection, Rubella, Sabia virus, Salmonella, Salmonella Paratyphi infection, Salmonella Typhi infection, Salmonellosis, SARS (severe acute respiratory syndrome), Scabies, Scarlet fever, Schistosomiasis, Scombroid, Sepsis, Septic Shock, Septicemic Plague, Severe Acute Respiratory Syndrome (SARS),
- 25 Shiga toxin-producing Escherichia coli, Shigella, Shigellosis, Shingles, Shingles (Herpes zoster), Smallpox, Sore Mouth (Orf Virus), Sporotrichosis, Spotted fever rickettsiosis, St. Louis encephalitis virus disease, Staphylococcal Infection, Staphylococcal Infection (Methicillin-resistant (MRSA)), Staphylococcal food poisoning, Staphylococcal Infection (Vancomycin Intermediate (VISA)), Strep Throat, Streptococcal Disease, Group A (invasive) (Strep A (invasive)),
- 30 Streptococcal Disease, Group B (Strep-B), Streptococcal toxic shock syndrome, Strongyloidiasis, Subacute sclerosing panencephalitis, Syphilis, Taeniasis, Tetanus Infection, Tickborne Diseases, Tinea barbae, Tinea capitis, Tinea corporis, Tinea cruris, Tinea manum, Tinea nigra, Tinea pedis, Tinea unguium, Tinea versicolor, Toxic shock syndrome, Toxocariasis (ocular larva migrans (OLM)), Toxocariasis (visceral larva migrans (VLM)),
- 35 Toxoplasmosis, Trachoma, Trichinellosis, Trichomoniasis, Trichonosis Infection (Trichinosis), Trichuriasis (whipworm infection), Tuberculosis (TB), Tularemia (Rabbit fever), Typhoid fever, Typhoid Fever, Group D, Typhus, Typhus fever, Ureaplasma urealyticum infection, Vaginosis,

Valley fever, Variant Creutzfeldt–Jakob disease (vCJD, nvCJD), Varicella (Chickenpox), Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, *Vibrio cholerae* (Cholera), *Vibrio parahaemolyticus* enteritis, *Vibrio vulnificus* infection, Vibriosis, Viral infection, Viral hemorrhagic fever, Viral Hemorrhagic Fever (Ebola, Lassa, Marburg), Viral Hemorrhagic Fevers (VHF), Viral pneumonia, West Nile virus disease, Western equine encephalitis virus disease, White piedra (tinea blanca), Whooping Cough, Yellow Fever, *Yersinia* (*Yersinia*), *Yersinia pseudotuberculosis* infection, Yersiniosis, Zeaspora, Zika fever, Zika Virus, Zika virus disease, congenital, Zika virus disease, non-congenital, Zika Virus Infection (Zika), Zika virus infection, congenital, Zika virus infection, non-congenital, and Zygomycosis. In various
5
10
embodiments, the infectious disease that can be treated by the disclosed methods and compositions is treated, while healthy cells are spared.

In one embodiment, the invention is a method of activating at least one $\gamma\delta$ T cell in a subject, comprising the step of administering an anti-V δ 1 antibody or fragment thereof as defined
15
herein.

In one embodiment, the invention is a method of causing or increasing proliferation of $\gamma\delta$ T cells in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.
20

In one embodiment, the invention is a method of causing or increasing the expansion of $\gamma\delta$ T cells in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

25 In one embodiment, the invention is a method of causing or increasing $\gamma\delta$ T cell degranulation in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

In one embodiment, the invention is a method of causing or increasing $\gamma\delta$ T cell killing activity
30
in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein. In one embodiment, the invention is a method of causing or increasing $\gamma\delta$ T cell killing activity in a subject, while sparing healthy cells, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

35 In one embodiment, the invention is a method of causing or increasing $\gamma\delta$ T cytotoxicity in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein. In one embodiment, the invention is a method of causing or

increasing $\gamma\delta$ T cytotoxicity in a subject, while sparing healthy cells, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

5 In one embodiment, the invention is a method of causing or increasing $\gamma\delta$ T cell mobilization in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

10 In one embodiment, the invention is a method of increasing survival of $\gamma\delta$ T cells in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

15 In one embodiment, the invention is a method of or increasing resistance to exhaustion of $\gamma\delta$ T cells in a subject, comprising the step of administering to the subject an anti-V δ 1 antibody or fragment thereof as defined herein.

According to a further aspect of the invention, there is provided a method of stimulating an immune response in a subject, the method comprising administration to the subject an anti-V δ 1 antibody or fragment thereof in an amount effective at stimulating an immune response.

20 **Uses of antibodies or fragments thereof**

According to a further aspect of the invention, there is provided the use of an anti-V δ 1 antibody or fragment thereof as described herein to study antigen recognition, activation, signal transduction or function of $\gamma\delta$ T cells (in particular V δ 1 T cells). As described herein, the antibodies have been shown to be active in assays which can be used to investigate $\gamma\delta$ T cell
25 function. Such antibodies may also be useful for inducing the proliferation of $\gamma\delta$ T cells, therefore may be used in methods of expanding $\gamma\delta$ T cells (such as V δ 1 T cells).

30 Antibodies which bind to the V δ 1 chain can be used to detect $\gamma\delta$ T cells. For example, the antibody may be labelled with a detectable label or reporter molecule or used as a capture ligand to selectively detect and/or isolate V δ 1 T cells in a sample. Labelled antibodies find use in many methods known in the art, for example immunohistochemistry and ELISA.

35 The detectable label or reporter molecule can be a radioisotope, such as ^3H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I ; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β -galactosidase, horseradish peroxidase, or luciferase. Fluorescent labels applied to antibodies of the invention may then be used in fluorescence-activated cell sorting (FACS) methods.

Thus in various embodiments, the invention includes *in vivo* methods of modulating $\gamma\delta$ T cells, methods of binding $\gamma\delta$ T cells, methods of targeting $\gamma\delta$ T cells, methods of activating $\gamma\delta$ T cells, methods of proliferating $\gamma\delta$ T cells, methods of expanding $\gamma\delta$ T cells, methods of detecting $\gamma\delta$ T cells, methods of causing $\gamma\delta$ T cell degranulation, methods of causing $\gamma\delta$ T cell killing activity, methods of selecting antibodies or fragments thereof, the methods comprising the step of administering an anti- $\gamma\delta$ antibody or fragment thereof to a subject as described herein.

CLAUSES

10 A set of clauses defining the invention and its preferred aspects is as follows:

1. A method of treating a cancer, an infectious disease or an inflammatory disease in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an anti-V δ 1 antibody or fragment thereof.
- 15 2. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises one or more of:
 - a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25;
 - 20 a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3); and/or
 - a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.
- 25 3. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13, such as SEQ ID NOs: 8, 9, 10 or 11.
4. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37, such as SEQ ID NOs: 32, 33, 34 or 35.
- 30 5. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-49, such as SEQ ID NOs: 44, 45, 46 or 47.
- 35

6. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 8, a CDR2 comprising a sequence of SEQ ID NO: 32, and a CDR1 comprising a sequence of SEQ ID NO: 44.
- 5 7. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 9, a CDR2 comprising a sequence of SEQ ID NO: 33, and a CDR1 comprising a sequence of SEQ ID NO: 45.
8. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises
10 a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 10, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR1 comprising a sequence of SEQ ID NO: 46.
9. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises
15 a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 11, a CDR2 comprising a sequence of SEQ ID NO: 35, and a CDR1 comprising a sequence of SEQ ID NO: 47.
10. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises
20 a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25, such as SEQ ID NOs: 20, 21, 22 or 23.
11. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises
25 a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12, such as SEQ ID NOs: A7, A8, A9 or A10.
12. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises
30 a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61, such as SEQ ID NOs: 56, 57, 58 or 59.
13. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises
a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 20, a CDR2 comprising a sequence of SEQUENCE: A7, and a CDR1 comprising a sequence of SEQ ID NO: 56.
- 35 14. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 21, a CDR2 comprising a sequence of SEQUENCE: A8, and a CDR1 comprising a sequence of SEQ ID NO: 57.

15. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 22, a CDR2 comprising a sequence of SEQUENCE: A9, and a CDR1 comprising a sequence of SEQ ID NO: 58.
- 5
16. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 23, a CDR2 comprising a sequence of SEQUENCE: A10, and a CDR1 comprising a sequence of SEQ ID NO: 59.
- 10
17. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 6 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 13.
18. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises CDR1, CDR2 and CDR3 sequences as defined in clause 7 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 14.
- 15
19. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises CDR1, CDR2 and CDR3 sequences as defined in clause 8 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 15.
- 20
20. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises CDR1, CDR2 and CDR3 sequences as defined in clause 9 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 16.
- 25
21. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-85.
- 30
22. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73.
- 35
23. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62, 63, 64, 68, 69, 70 or 71.

24. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85.
- 5 25. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74, 75, 76, 80, 81, 82 or 83.
- 10 26. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 62 and a VL region comprising an amino acid sequence of SEQ ID NO: 74.
- 15 27. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 63 and a VL region comprising an amino acid sequence of SEQ ID NO: 75.
- 20 28. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 64 and a VL region comprising an amino acid sequence of SEQ ID NO: 76.
- 25 29. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 68 and a VL region comprising an amino acid sequence of SEQ ID NO: 80.
- 30 30. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 69 and a VL region comprising an amino acid sequence of SEQ ID NO: 81.
31. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 70 and a VL region comprising an amino acid sequence of SEQ ID NO: 82.
- 35 32. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 71 and a VL region comprising an amino acid sequence of SEQ ID NO: 83.

33. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises a VH region and a VL region, wherein the VH and VL region are joined by a linker, such as a polypeptide linker.
- 5 34. The method of clause 33, wherein the linker comprises a (Gly4Ser) n format, where n = 1 to 8
35. The method of clause 33 or clause 34, wherein the linker comprises a [(Gly4Ser) n (Gly3AlaSer) m] p linker, where n , m and p = 1 to 8.
- 10 36. The method of clause 33 or clause 35, wherein the linker comprises SEQ ID NO: 98.
37. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs:
- 15 86-97.
38. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises an amino acid sequence of any one of SEQ ID NOs: 86-97.
- 20 39. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 86.
40. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 87.
- 25 41. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 88.
42. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises
- 30 SEQ ID NO: 92.
43. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 93.
- 35 44. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 94.

45. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 95.
46. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof binds to the same, or essentially the same, epitope as, or competes with, an antibody or fragment thereof as defined in any one of clauses 1 to 45.
47. The method of clause 1, wherein the anti-V δ 1 antibody or fragment thereof comprises binds to an epitope of a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR) comprising one or more amino acid residues within amino acids 1-90 of SEQ ID NO: 1.
48. The method of clause 47, wherein the epitope comprises at least one of amino acid residues 3, 5, 9, 10, 12, 16, 17, 20, 37, 42, 50, 53, 59, 62, 64, 68, 69, 72 or 77 of SEQ ID NO: 1.
49. The method of clauses 47 or 48, wherein the epitope comprises one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69, of SEQ ID NO: 1.
50. The method of any one of clauses 47 to 49, wherein the epitope consists of one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69 of SEQ ID NO: 1.
51. The method of any one of clauses 47 to 50, wherein the epitope comprises one or more amino acid residues within amino acid regions 5-20 and 62-77 of SEQ ID NO: 1.
52. The method of any one of clauses 47 to 51, wherein the epitope comprises one or more amino acid residues within amino acid region 50-64 of SEQ ID NO: 1.
53. The method of any one of clauses 47 to 52, wherein the epitope comprises one or more amino acid residues within amino acid regions 37-53 and 59-77 of SEQ ID NO: 1.
54. The method of any one of clauses 47 to 53, wherein the epitope is an activating epitope of a $\gamma\delta$ T cell.
55. The method of clause 54, wherein binding of the activating epitope: (i) downregulates the $\gamma\delta$ TCR; (ii) activates degranulation of the $\gamma\delta$ T cell; and/or (iii) activates $\gamma\delta$ T cell killing.

56. The method of any one of clauses 47 to 55, wherein the antibody or fragment thereof only binds to an epitope in the V region of a V δ 1 chain of a $\gamma\delta$ TCR.
- 5 57. The method of any one of clauses 47 to 56, wherein the antibody or fragment thereof does not bind to an epitope found in CDR3 of a V δ 1 chain of a $\gamma\delta$ TCR.
58. The method of clause 57, wherein the antibody or fragment thereof does not bind to an epitope within amino acid region 91-105 (CDR3) of SEQ ID NO: 1.
- 10 59. The method of any one of clauses 1 to 58, wherein the antibody or fragment thereof binds a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR) with a binding affinity (KD) as measured by surface plasmon resonance of less than 1.5×10^{-7} M.
- 15 60. The method of clause 59, wherein antibody or fragment thereof exhibits a KD less than 1.3×10^{-7} M or less, such as less than 1.0×10^{-7} M, in particular less than 5.0×10^{-8} M.
61. The method of clause 1, wherein the antibody or fragment thereof has an EC50 value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.5 μ g/ml.
- 20 62. The method of clause 1, wherein the antibody or fragment thereof has an EC50 value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.06 μ g/ml.
63. The method of clause 1, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.05 μ g/ml.
- 25 64. The method of clause 1, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.005 μ g/ml, such as less than 0.002 μ g/ml.
- 30 65. The method of clause 63 or clause 64, wherein the $\gamma\delta$ T cell degranulation EC50 value is measured by detecting CD107a expression.
66. The method of clause 1, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.5 μ g/ml.
- 35

67. The method of clause 1, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.055 $\mu\text{g/ml}$, such as less than 0.020 $\mu\text{g/ml}$.

5 68. The method of any one of clauses 61 to 67, wherein the EC50 value is measured using flow cytometry.

69. The method of clause 1, wherein antibody or fragment thereof as defined in any one of clauses 1 to 68, is an scFv, Fab, Fab', F(ab')₂, Fv, variable domain (e.g. VH or VL), diabody, minibody or full length antibody.
10

70. The method of clause 69, wherein the antibody or fragment thereof is an scFv or a full length antibody, such as IgG1.

71. The method of clause 70, wherein the antibody or fragment thereof comprises an amino acid sequence having at least 80% sequence identity with of any one of SEQ ID NOs: 111-122.
15

72. The method of clause 70 or clause 71, wherein the antibody or fragment thereof comprises an amino acid sequence of any one of SEQ ID NOs: 111-122.
20

73. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 111.

74. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 112.
25

75. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 116.

30 76. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 117.

77. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 118.
35

78. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 119.

79. The method of clause 71 or clause 72, wherein the antibody or fragment thereof comprises SEQ ID NO: 120.
- 5 80. The method of any one of clause 1 or clause 79, wherein the antibody or fragment thereof is human.
81. The method of clause 1, wherein the anti- $\gamma\delta$ 1 antibody or fragment thereof with an EC50 value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.5 $\mu\text{g/ml}$; an
- 10 EC50 value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.05 $\mu\text{g/ml}$; and/or an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.5 $\mu\text{g/ml}$.
82. A method of modulating an immune response in a subject in need thereof, comprising the step of administering to the subject an anti- $\gamma\delta$ 1 antibody or fragment thereof as defined in
- 15 any one of clauses 1 to 81.
83. The method of clause 82, wherein the subject has cancer, an infectious disease or an inflammatory disease.
- 20 84. The method of clause 82, wherein modulating an immune response in a subject comprises at least one selected from the group consisting of activating $\gamma\delta$ T cells, causing or increasing proliferation $\gamma\delta$ T cells, causing or increasing expansion of $\gamma\delta$ T cells, causing or increasing $\gamma\delta$ T cell degranulation, causing or increasing $\gamma\delta$ T cell killing activity, causing or increasing $\gamma\delta$ T cytotoxicity, causing or increasing $\gamma\delta$ T cell mobilization, increasing survival of
- 25 $\gamma\delta$ T cells, and increasing resistance to exhaustion of $\gamma\delta$ T cells.
85. The method of any one of clauses 1 to 84, wherein diseased cells are killed while healthy cells are spared.
- 30 86. An isolated multi-specific antibody or fragment thereof that binds to at least two target antigens, wherein the first of the at least two target antigens is V δ 1, and which comprises one or more of:
- a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25;
- 35 a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3); and/or

a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.

87. The isolated multi-specific antibody or fragment thereof as defined in clause 86, which
5 comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13, such as SEQ ID NOs: 8, 9, 10 or 11.

88. The isolated multi-specific antibody or fragment thereof as defined in clause 86 or
10 clause 87, which comprises a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37, such as SEQ ID NOs: 32, 33, 34 or 35.

89. The isolated multi-specific antibody or fragment thereof as defined in any one of
15 clauses 86 to 88, which comprises a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-49, such as SEQ ID NOs: 44, 45, 46 or 47.

90. The isolated multi-specific antibody or fragment thereof as defined in any one of
20 clauses 86 to 89, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 8, a CDR2 comprising a sequence of SEQ ID NO: 32, and a CDR1 comprising a sequence of SEQ ID NO: 44.

91. The isolated multi-specific antibody or fragment thereof as defined in any one of
25 clauses 86 to 89, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 9, a CDR2 comprising a sequence of SEQ ID NO: 33, and a CDR1 comprising a sequence of SEQ ID NO: 45.

92. The isolated multi-specific antibody or fragment thereof as defined in any one of
30 clauses 86 to 89, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 10, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR1 comprising a sequence of SEQ ID NO: 46.

93. The isolated multi-specific antibody or fragment thereof as defined in any one of
35 clauses 86 to 89, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 11, a CDR2 comprising a sequence of SEQ ID NO: 35, and a CDR1 comprising a sequence of SEQ ID NO: 47.

94. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 93, which comprises a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25, such as SEQ ID NOs: 20, 21, 22 or 23.

5

95. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 94, which comprises a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12, such as SEQ ID NOs: A7, A8, A9 or A10.

10

96. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 95, which comprises a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61, such as SEQ ID NOs: 56, 57, 58 or 59.

15

97. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 95, which comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 20, a CDR2 comprising a sequence of SEQUENCE: A7, and a CDR1 comprising a sequence of SEQ ID NO: 56.

20

98. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 95, which comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 21, a CDR2 comprising a sequence of SEQUENCE: A8, and a CDR1 comprising a sequence of SEQ ID NO: 57.

25

99. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 95, which comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 22, a CDR2 comprising a sequence of SEQUENCE: A9, and a CDR1 comprising a sequence of SEQ ID NO: 58.

30

100. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 95, which comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 23, a CDR2 comprising a sequence of SEQUENCE: A10, and a CDR1 comprising a sequence of SEQ ID NO: 59.

35

101. An isolated multi-specific antibody or fragment thereof which comprises a VH region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 90 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 97.

- 5 102. An isolated multi-specific antibody or fragment thereof which comprises a VH region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 91 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 98.

- 10 103. An isolated multi-specific antibody or fragment thereof which comprises a VH region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 92 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 100.

- 15 104. An isolated multi-specific antibody or fragment thereof which comprises a VH region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 93 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 100.

105. An isolated multi-specific antibody or fragment thereof, which comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-85.

- 20 106. The isolated multi-specific antibody or fragment thereof as defined in clause 49, which comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73.

- 25 107. The isolated multi-specific antibody or fragment thereof as defined in clause 50, wherein the VH region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62, 63, 64, 68, 69, 70 or 71.

- 30 108. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 107, which comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85.

109. The isolated multi-specific antibody or fragment thereof as defined in clause 108, wherein the VL region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74, 75, 76, 80, 81, 82 or 83.

110. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 62 and a VL region comprising an amino acid sequence of SEQ ID NO: 74.

- 5 111. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 63 and a VL region comprising an amino acid sequence of SEQ ID NO: 75.

- 10 112. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 64 and a VL region comprising an amino acid sequence of SEQ ID NO: 76.

- 15 113. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 68 and a VL region comprising an amino acid sequence of SEQ ID NO: 80.

- 20 114. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 69 and a VL region comprising an amino acid sequence of SEQ ID NO: 81.

115. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 70 and a VL region comprising an amino acid sequence of SEQ ID NO: 82.

- 25 116. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 109, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 71 and a VL region comprising an amino acid sequence of SEQ ID NO: 83.

- 30 117. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 105 to 116, wherein the VH and VL region are joined by a linker, such as a polypeptide linker.

118. The isolated multi-specific antibody or fragment thereof as defined in clause 117, wherein the linker comprises a (Gly4Ser)_n format, where n = 1 to 8

119. The isolated multi-specific antibody or fragment thereof as defined in clause 117 or clause 118, wherein the linker comprises a [(Gly4Ser)_n(Gly3AlaSer)_m]_p linker, where n, m and p = 1 to 8.
- 5 120. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 117 to 118, wherein the linker comprises SEQ ID NO: 98.
121. The isolated multi-specific antibody or fragment thereof as defined in clause 120, wherein the linker consists of SEQ ID NO: 98.
- 10 122. An isolated multi-specific antibody or fragment thereof which comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 86-97.
123. The isolated multi-specific antibody or fragment thereof as defined in clause 122, which
15 comprises an amino acid sequence of any one of SEQ ID NOs: 86-97.
124. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or clause 122, which comprises SEQ ID NO: 86.
- 20 125. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or clause 122, which comprises SEQ ID NO: 87.
126. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or clause 122, which comprises SEQ ID NO: 88.
- 25 127. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or clause 122, which comprises SEQ ID NO: 92.
128. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or
30 clause 122, which comprises SEQ ID NO: 93.
129. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or clause 122, which comprises SEQ ID NO: 94.
- 35 130. The isolated multi-specific antibody or fragment thereof as defined in clause 94 or clause 122, which comprises SEQ ID NO: 95.

131. An isolated multi-specific antibody or fragment thereof, which binds to the same, or essentially the same, V δ 1 epitope as, or competes with, an antibody or fragment thereof as defined in any one of clauses 86 to 130.

- 5 132. A human, isolated anti-TCR delta variable 1, multi-specific antibody or fragment thereof, that binds to at least two target antigens, wherein the first of the at least two target antigens is V δ 1, and wherein the multi-specific antibody or fragment thereof which binds to an epitope of V δ 1 comprising one or more amino acid residues within amino acids 1-90 of SEQ ID NO: 1.

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133. The human, isolated multi-specific antibody or fragment thereof as defined in clause 132, wherein the epitope comprises at least one of amino acid residues 3, 5, 9, 10, 12, 16, 17, 20, 37, 42, 50, 53, 59, 62, 64, 68, 69, 72 or 77 of SEQ ID NO: 1.

- 15 134. The human, isolated multi-specific antibody or fragment thereof as defined in clause 132 or clause 133, wherein the epitope comprises one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69, of SEQ ID NO: 1.

- 20 135. The human, isolated multi-specific antibody or fragment thereof as defined in clause 134, wherein the epitope consists of one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69 of SEQ ID NO: 1.

- 25 136. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 132 to 135, wherein the epitope comprises one or more amino acid residues within amino acid regions 5-20 and 62-77 of SEQ ID NO: 1.

- 30 137. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 132 to 135, wherein the epitope comprises one or more amino acid residues within amino acid region 50-64 of SEQ ID NO: 1.

- 35 138. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 132 to 135, wherein the epitope comprises one or more amino acid residues within amino acid regions 37-53 and 59-77 of SEQ ID NO: 1.

139. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 132 to 138, wherein the epitope is an activating epitope of a $\gamma\delta$ T cell.

140. The human, isolated multi-specific antibody or fragment thereof as defined in clause 139, wherein binding of the activating epitope: (i) downregulates the $\gamma\delta$ TCR; (ii) activates degranulation of the $\gamma\delta$ T cell; and/or (iii) activates $\gamma\delta$ T cell killing.

5

141. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 132 to 140, which only binds to an epitope in the V region of a V δ 1 chain of a $\gamma\delta$ TCR.

10 142. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 132 to 141, which does not bind to an epitope found in CDR3 of a V δ 1 chain of a $\gamma\delta$ TCR.

143. The human isolated multi-specific antibody or fragment thereof as defined in clause
15 142, which does not bind to an epitope within amino acid region 91-105 (CDR3) of SEQ ID NO: 1.

144. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 143, which binds a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR)
20 with a binding affinity (KD) as measured by surface plasmon resonance of less than 1.5×10^{-7} M.

145. The isolated multi-specific antibody or fragment thereof as defined in clause 144, wherein the KD is less than 1.3×10^{-7} M or less, such as less than 1.0×10^{-7} M, in particular
25 less than 5.0×10^{-8} M.

146. An isolated multi-specific antibody or fragment thereof with an EC₅₀ value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.5 $\mu\text{g/ml}$.

30 147. An isolated multi-specific antibody or fragment thereof with an EC₅₀ value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.06 $\mu\text{g/ml}$.

148. An isolated multi-specific antibody or fragment thereof with an EC₅₀ value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.05 $\mu\text{g/ml}$.

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149. An isolated multi-specific antibody or fragment thereof with an EC₅₀ value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.005 $\mu\text{g/ml}$, such as less than 0.002 $\mu\text{g/ml}$.

150. The isolated multi-specific antibody or fragment thereof as defined in clause 148 or clause 149, wherein the $\gamma\delta$ T cell degranulation EC50 value is measured by detecting CD107a expression.

5

151. An isolated multi-specific antibody or fragment thereof with an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.5 $\mu\text{g/ml}$.

152. An isolated multi-specific antibody or fragment thereof with an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.055 $\mu\text{g/ml}$, such as less than 0.020 $\mu\text{g/ml}$.

10

153. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 146 to 152, wherein the EC50 value is measured using flow cytometry.

154. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 153, which is an scFv, Fab, Fab', F(ab')₂, Fv, variable domain (e.g. VH or VL), diabody, minibody or full length antibody.

15

155. The isolated multi-specific antibody or fragment thereof as defined in clause 154, which is an scFv or a full length antibody, such as IgG1.

20

156. The isolated multi-specific antibody as defined in clause 155, which comprises an amino acid sequence having at least 80% sequence identity with of any one of SEQ ID NOs: 111-122.

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157. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises an amino acid sequence of any one of SEQ ID NOs: 111-122.

158. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises SEQ ID NO: 111.

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159. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises SEQ ID NO: 112.

160. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises SEQ ID NO: 116.

35

161. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises SEQ ID NO: 117.

162. The isolated multi-specific antibody as defined in clause 155 or clause 156, which
5 comprises SEQ ID NO: 118.

163. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises SEQ ID NO: 119.

10 164. The isolated multi-specific antibody as defined in clause 155 or clause 156, which comprises SEQ ID NO: 120.

165. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 164, which is human.

15

166. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 165, wherein the second of the at least two target antigens is one selected from the group consisting of CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3, CD3d, CD3e, CD3g, CD4, CD5, CD6, CD7, CD8, CD8a, CD8b, CD9, CD10, CD11a, CD11b, CD11c, CD11d,
20 CD13, CD14, CD15, CD16, CD16a, CD16b, CD17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32A, CD32B, CD33, CD34, CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60a, CD60b, CD60c, CD61,
25 CD62E, CD62L, CD62P, CD63, CD64a, CD65, CD65s, CD66a, CD66b, CD66c, CD66d, CD66e, CD66f, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD77, CD79A, CD79B, CD80, CD81, CD82, CD83, CD84, CD85A, CD85B, CD85C, CD85D, CD85F, CD85G, CD85H, CD85I, CD85J, CD85K, CD85M, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103,
30 CD104, CD105, CD106, CD107, CD107a, CD107b, CD108, CD109, CD110, CD111, CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120, CD120a, CD120b, CD121a, CD121b, CD122, CD123, CD124, CD125, CD126, CD127, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140A, CD140B, CD141, CD142, CD143, CD144, CDw145, CD146, CD147, CD148, CD150, CD151, CD152, CD153,
35 CD154, CD155, CD156, CD156a, CD156b, CD156c, CD157, CD158, CD158A, CD158B1, CD158B2, CD158C, CD158D, CD158E1, CD158E2, CD158F1, CD158F2, CD158G, CD158H, CD158I, CD158J, CD158K, CD159a, CD159c, CD160, CD161, CD162, CD163,

CD164, CD165, CD166, CD167a, CD167b, CD168, CD169, CD170, CD171, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179a, CD179b, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CD187, CD188, CD189, CD190, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CDw198, CDw199, CD200, 5 CD201, CD202b, CD203c, CD204, CD205, CD206, CD207, CD208, CD209, CD210, CDw210a, CDw210b, CD211, CD212, CD213a1, CD213a2, CD214, CD215, CD216, CD217, CD218a, CD218b, CD219, CD220, CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235a, CD235b, CD236, CD237, CD238, CD239, CD240CE, CD240D, CD241, CD242, CD243, CD244, CD245[17], CD246, 10 CD247, CD248, CD249, CD250, CD251, CD252, CD253, CD254, CD255, CD256, CD257, CD258, CD259, CD260, CD261, CD262, CD263, CD264, CD265, CD266, CD267, CD268, CD269, CD270, CD271, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD280, CD281, CD282, CD283, CD284, CD285, CD286, CD287, CD288, CD289, CD290, CD291, CD292, CDw293, CD294, CD295, CD296, CD297, CD298, CD299, CD300A, 15 CD300C, CD301, CD302, CD303, CD304, CD305, CD306, CD307, CD307a, CD307b, CD307c, CD307d, CD307e, CD308, CD309, CD310, CD311, CD312, CD313, CD314, CD315, CD316, CD317, CD318, CD319, CD320, CD321, CD322, CD323, CD324, CD325, CD326, CD327, CD328, CD329, CD330, CD331, CD332, CD333, CD334, CD335, CD336, CD337, CD338, CD339, CD340, CD344, CD349, CD351, CD352, CD353, CD354, CD355, CD357, 20 CD358, CD360, CD361, CD362, CD363, CD364, CD365, CD366, CD367, CD368, CD369, CD370, and CD371.

167. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 165, wherein the second of the at least two target antigens is one selected from 25 the group consisting of AFP, AKAP-4, ALK, alphafetoprotein, Androgen receptor, B7H3, BAGE, BCA225, BCAA, Bcr-abl, beta-Catenin, beta-HCG, beta-human chorionic gonadotropin, BORIS, BTAA, CA 125, CA 15-3, CA 195, CA 19-9, CA 242, CA 27.29, CA 72-4, CA-50, CAM 17.1, CAM43, Carbonic anhydrase IX, carcinoembryonic antigen, CD22, CD33/IL3Ra, CD68\IP1, CDK4, CEA, chondroitin sulfate proteoglycan 4 (CSPG4), c-Met, CO- 30 029, CSPG4, Cyclin B1, cyclophilin C-associated protein, CYP1B1, E2A-PRL, EGFR, EGFRvIII, ELF2M, EpCAM, EphA2, EphrinB2, Epstein Barr virus antigens EBVA, ERG (TMPRSS2ETS fusion gene), ETV6-AML, FAP, FGF-5, Fos-related antigen 1, Fucosyl GM1, G250, Ga733\EpCAM, GAGE-1, GAGE-2, GD2, GD3, glioma-associated antigen, GloboH, Glycolipid F77, GM3, GP 100, GP 100 (Pmel 17), H4-RET, HER-2/neu, HER-2/Neu/ErbB-2, 35 high-molecular-weight melanoma-associated antigen (HMW-MAA), HPV E6, HPV E7, hTERT, HTgp-175, human telomerase reverse transcriptase, Idiotype, IGF-I receptor, IGF-II, IGH-IGK, insulin growth factor (IGF)-I, intestinal carboxyl esterase, K-ras, LAGE-1a, LCK, lectin-

reactive AFP, Legumain, LMP2, M344, MA-50, Mac-2 binding protein, MAD-CT-1, MAD-CT-2, MAGE, MAGE A1, MAGE A3, MAGE-1, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MART-1, MART-1/MelanA, M-CSF, melanoma-associated chondroitin sulfate proteoglycan (MCSP), Mesothelin, MG7-Ag, ML-IAP, MN-CA IX, MOV18, MUC1, Mum-1, hsp70-2, MYCN, MYL-RAR, NA17, NB/70K, neuron-glia antigen 2 (NG2), neutrophil elastase, nm-23H1, NuMa, NY-BR-1, NY-CO-1, NY-ESO, NY-ESO-1, NY-ESO-1, OY-TES1, p15, p16, p180erbB3, p185erbB2, p53, p53 mutant, Page4, PAX3, PAX5, PDGFR-beta, PLAC1, Polysialic Acid, prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen, prostatic acid phosphatase (PAP), Proteinase3 (PR1), PSA, PSCA, PSMA, RAGE-1, Ras, Ras-mutant, RCAS1, RGS5, RhoC, ROR1, RU1, RU2 (AS), SART3, SDCCAG16, sLe(a), Sperm protein 17, SSX2, STn, Survivin, TA-90, TAAL6, a TAG-72, telomerase, thyroglobulin, Tie 2, TIGIT, TLP, Tn, TPS, TRP-1, TRP-2, TRP-2, TSP-180, Tyrosinase, VEGFR2, VISTA, WT1, XAGE 1, 43-9F, 5T4, and 791Tgp72.

168. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 165, wherein the multi-specific antibody format is one selected from the groups consisting of CrossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, Knobs-in-holes (KIH), Knobs-in-holes (common light chain), Charge pair, Fab-arm exchange, SEEDbody, Triomab, LUZ-Y, Fcab, $\kappa\lambda$ -body, orthogonal Fab, DVD-IgG, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zyboby, DVI-IgG(four-in-one), Nanobody, Nanoby-HAS, BiTE, Diabody, DART, TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, Triple Body, Miniantibody, Minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFV-CH-CL-scFv, F(ab')₂, F(ab')₂-scFv2, scFv-KIH, Fab-scFv-Fc, Tetravalent HCAb, scDiabody-Fc, Diabody-Fc, Tandem scFv-Fc, Intrabody, Dock and Lock, ImmTAC, HSAbody, scDiabody-HAS, Tandem scFv-Toxin, IgG-IgG, ov-X-Body, duobody, mab2 and scFv1-PEG-scFv2.

169. A polynucleotide sequence encoding the multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 168.

30

170. A polynucleotide sequence encoding the multi-specific antibody or fragment thereof comprising a sequence having at least 70% sequence identity with SEQ ID NO: 99-110.

171. A polynucleotide sequence encoding the multi-specific antibody or fragment thereof comprising a sequence of SEQ ID NO: 99-110.

35

172. An expression vector encoding the multi-specific antibody or fragment thereof comprising the polynucleotide sequence as defined in any one of clauses 169 to 171.

173. An expression vector encoding the multi-specific antibody or fragment thereof comprising the VH region of SEQ ID NO: 99-110.

174. An expression vector encoding the multi-specific antibody or fragment thereof comprising the VL region of SEQ ID NO: 99-110.

175. An expression vector encoding the multi-specific antibody or fragment thereof comprising the VH region of clause 173 and the VL region of clause 174.

176. A cell comprising the polynucleotide sequence encoding the multi-specific antibody or fragment thereof as defined in any one of clauses 169 to 171 or the expression vector as defined in any one of clauses 172 to 175.

177. A cell comprising a first expression vector encoding the multi-specific antibody or fragment thereof as defined in clause 173 and a second expression vector encoding the multi-specific antibody or fragment thereof as defined in clause 174.

178. A cell comprising the expression vector encoding the multi-specific antibody or fragment thereof as defined in clause 175.

179. The cell as defined in any one of clauses 176 to 178, wherein the polynucleotide or expression vector encoding the multi-specific antibody or fragment thereof encodes a membrane anchor or transmembrane domain fused to the antibody or fragment thereof, wherein the antibody or fragment thereof is presented on an extracellular surface of the cell.

180. A composition comprising the multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 168.

181. A pharmaceutical composition comprising the multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 168, together with a pharmaceutically acceptable diluent or carrier.

182. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 168 or the pharmaceutical composition as defined in clause 125, for use as a medicament.

- 5 183. The isolated multi-specific antibody or fragment thereof or the pharmaceutical composition as defined in clause 182 for use in the treatment of cancer, an infectious disease or an inflammatory disease.

- 10 184. A method of treating a cancer, an infectious disease or an inflammatory disease in a subject in need thereof, comprising administering a therapeutically effective amount of the isolated multi-specific antibody or fragment thereof as defined in any one of clauses 86 to 168 or the pharmaceutical composition as defined in clause 125.

- 15 184. An isolated antigen comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO: 123 for use in generating a multi-specific antibody or fragment thereof.

185. A method of generating a multi-specific antibody or fragment thereof comprising:
- 20 (i) designing a series of antigens comprising a TCR delta variable 1 (TRDV1) amino acid sequence wherein the CDR3 sequence of the TRDV1 is the same for all antigens in the series;
- (ii) exposing a first antigen designed in step (i) to an antibody library;
- (iii) isolating the antibodies or fragments thereof which bind to the antigen;
- (iv) exposing the isolated antibodies or fragments thereof to a second antigen designed in step (i); and
- 25 (v) isolating the antibodies or fragments thereof which bind to both the first and second antigen.

186. The method as defined in clause 185, which further comprises exposing the isolated antibodies or fragments thereof to a second series of antigens comprising a $\gamma\delta$ TCR with a different delta variable chain, such as TCR delta variable 2 (TRDV2) or TCR delta variable 3 (TRDV3), and then deselecting the antibodies or fragments thereof which also bind to the second series of antigens.
- 30

187. The method as defined in clause 185 or clause 186, wherein the first and/or second series of antigens are presented as a leucine zipper and/or Fc fusion.
- 35

188. The method as defined in any one of clauses 185 to 187, wherein the series of antigens are in a heterodimeric and/or homodimeric format.

189. An antibody obtained by the method as defined in any one of clauses 185 to 188.

5

190. An anti-vδ1 antibody or fragment thereof for use in a method of treating cancer, an infectious disease or an inflammatory disease.

191. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises one or more of:

10 a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25;

a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3); and/or

15 a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.

192. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13, such as SEQ ID NOs: 8, 9, 10 or 11.

193. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37, such as SEQ ID NOs: 32, 33, 34 or 35.

194. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-49, such as SEQ ID NOs: 44, 45, 46 or 47.

195. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 8, a CDR2 comprising a sequence of SEQ ID NO: 32, and a CDR1 comprising a sequence of SEQ ID NO: 44

196. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 9, a CDR2 comprising a sequence of SEQ ID NO: 33, and a CDR1 comprising a sequence of SEQ ID NO: 45.

5

197. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 10, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR1 comprising a sequence of SEQ ID NO: 46.

10

198. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 11, a CDR2 comprising a sequence of SEQ ID NO: 35, and a CDR1 comprising a sequence of SEQ ID NO: 47.

15

199. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25, such as SEQ ID NOs: 20, 21, 22 or 23.

20

200. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12, such as SEQ ID NOs: A7, A8, A9 or A10.

25

201. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61, such as SEQ ID NOs: 56, 57, 58 or 59.

30

202. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of SEQ ID NO: 20, a CDR2 comprising a sequence of SEQUENCE: A7, and a CDR1 comprising a sequence of SEQ ID NO: 56.

35

203. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of

SEQ ID NO: 21, a CDR2 comprising a sequence of SEQUENCE: A8, and a CDR1 comprising a sequence of SEQ ID NO: 57.

204. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
5 or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of
SEQ ID NO: 22, a CDR2 comprising a sequence of SEQUENCE: A9, and a CDR1 comprising
a sequence of SEQ ID NO: 58.

205. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
10 or fragment thereof, comprises a VL region comprising a CDR3 comprising a sequence of
SEQ ID NO: 23, a CDR2 comprising a sequence of SEQUENCE: A10, and a CDR1 comprising
a sequence of SEQ ID NO: 59.

206. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
15 or fragment thereof, comprises a VH region comprising CDR1, CDR2 and CDR3 sequences
as defined in clause 6 and a VL region comprising CDR1, CDR2 and CDR3 sequences as
defined in clause 202.

207. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
20 or fragment thereof, comprises CDR1, CDR2 and CDR3 sequences as defined in clause 7
and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 203.

208. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
or fragment thereof, comprises CDR1, CDR2 and CDR3 sequences as defined in clause 8
25 and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 204.

209. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
or fragment thereof, comprises CDR1, CDR2 and CDR3 sequences as defined in clause 9
and a VL region comprising CDR1, CDR2 and CDR3 sequences as defined in clause 205.
30

210. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
or fragment thereof, comprises an amino acid sequence having at least 80% sequence identity
with any one of SEQ ID NOs: 62-85.

35 211. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
or fragment thereof, comprises a VH region comprising an amino acid sequence having at
least 80% sequence identity with any one of SEQ ID NOs: 62-73.

212. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62, 63, 64, 68, 69, 70 or 71.
- 5
213. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85.
- 10
214. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74, 75, 76, 80, 81, 82 or 83.
215. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 62 and a VL region comprising an amino acid sequence of SEQ ID NO: 74.
- 15
216. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 63 and a VL region comprising an amino acid sequence of SEQ ID NO: 75.
- 20
217. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 64 and a VL region comprising an amino acid sequence of SEQ ID NO: 76.
- 25
218. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 68 and a VL region comprising an amino acid sequence of SEQ ID NO: 80.
- 30
219. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 69 and a VL region comprising an amino acid sequence of SEQ ID NO: 81.
- 35
220. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 70 and a VL region comprising an amino acid sequence of SEQ ID NO: 82.

221. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof, comprises a VH region comprising an amino acid sequence of SEQ ID NO: 71 and a VL region comprising an amino acid sequence of SEQ ID NO: 83.
- 5 222. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof comprises a VH region and a VL region, wherein the VH and VL region are joined by a linker, such as a polypeptide linker.
223. The anti-vδ1 antibody or fragment thereof of clause 222, wherein the linker comprises
10 a (Gly4Ser)_n format, where n = 1 to 8
224. The anti-vδ1 antibody or fragment thereof of clause 222 or clause 223, wherein the linker comprises a [(Gly4Ser)_n(Gly3AlaSer)_m]_p linker, where n, m and p = 1 to 8.
- 15 225. The anti-vδ1 antibody or fragment thereof of clause 222 or clause 224, wherein the linker comprises SEQ ID NO: 98.
226. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof comprises an amino acid sequence having at least 80% sequence identity
20 with any one of SEQ ID NOs: 86-97.
227. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof comprises an amino acid sequence of any one of SEQ ID NOs: 86-97.
- 25 228. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof comprises SEQ ID NO: 86.
229. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof comprises SEQ ID NO: 87.
30
230. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody or fragment thereof comprises SEQ ID NO: 88.
231. The anti-vδ1 antibody or fragment thereof of clause 190, wherein the anti-Vδ1 antibody
35 or fragment thereof comprises SEQ ID NO: 92.

232. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 93.
233. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
5 or fragment thereof comprises SEQ ID NO: 94.
234. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody or fragment thereof comprises SEQ ID NO: 95.
- 10 235. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody or fragment thereof binds to the same, or essentially the same, epitope as, or competes with, an antibody or fragment thereof as defined in any one of clauses 190 to 234.
236. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the anti-V δ 1 antibody
15 or fragment thereof comprises binds to an epitope of a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR) comprising one or more amino acid residues within amino acids 1-90 of SEQ ID NO: 1.
237. The anti-v δ 1 antibody or fragment thereof of clause 236, wherein the epitope
20 comprises at least one of amino acid residues 3, 5, 9, 10, 12, 16, 17, 20, 37, 42, 50, 53, 59, 62, 64, 68, 69, 72 or 77 of SEQ ID NO: 1.
238. The anti-v δ 1 antibody or fragment thereof of clauses 236 or 237, wherein the epitope
25 comprises one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69, of SEQ ID NO: 1.
239. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 238, wherein
30 the epitope consists of one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69 of SEQ ID NO: 1.
240. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 239, wherein
the epitope comprises one or more amino acid residues within amino acid regions 5-20 and 62-77 of SEQ ID NO: 1.
- 35 241. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 240, wherein the epitope comprises one or more amino acid residues within amino acid region 50-64 of SEQ ID NO: 1.

242. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 241, wherein the epitope comprises one or more amino acid residues within amino acid regions 37-53 and 59-77 of SEQ ID NO: 1.

5

243. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 242, wherein the epitope is an activating epitope of a $\gamma\delta$ T cell.

244. The anti-v δ 1 antibody or fragment thereof of clause 243, wherein binding of the activating epitope: (i) downregulates the $\gamma\delta$ TCR; (ii) activates degranulation of the $\gamma\delta$ T cell; and/or (iii) activates $\gamma\delta$ T cell killing.

245. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 244, wherein the antibody or fragment thereof only binds to an epitope in the V region of a V δ 1 chain of a $\gamma\delta$ TCR.

246. The anti-v δ 1 antibody or fragment thereof of any one of clauses 236 to 245, wherein the antibody or fragment thereof does not bind to an epitope found in CDR3 of a V δ 1 chain of a $\gamma\delta$ TCR.

20

247. The anti-v δ 1 antibody or fragment thereof of clause 246, wherein the antibody or fragment thereof does not bind to an epitope within amino acid region 91-105 (CDR3) of SEQ ID NO: 1.

248. The anti-v δ 1 antibody or fragment thereof of any one of clauses 190 to 247, wherein the antibody or fragment thereof binds a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR) with a binding affinity (KD) as measured by surface plasmon resonance of less than 1.5×10^{-7} M.

249. The anti-v δ 1 antibody or fragment thereof of clause 248, wherein antibody or fragment thereof exhibits a KD less than 1.3×10^{-7} M or less, such as less than 1.0×10^{-7} M, in particular less than 5.0×10^{-8} M.

250. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the antibody or fragment thereof has an EC50 value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.5 μ g/ml.

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251. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the antibody or fragment thereof has an EC50 value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.06 μ g/ml.

5 252. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.05 μ g/ml.

10 253. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.005 μ g/ml, such as less than 0.002 μ g/ml.

15 254. The anti-v δ 1 antibody or fragment thereof of clause 252 or clause 253, wherein the $\gamma\delta$ T cell degranulation EC50 value is measured by detecting CD107a expression.

255. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.5 μ g/ml.

20 256. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein the antibody or fragment thereof has an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.055 μ g/ml, such as less than 0.020 μ g/ml.

25 257. The anti-v δ 1 antibody or fragment thereof of any one of clauses 250 to 256, wherein the EC50 value is measured using flow cytometry.

258. The anti-v δ 1 antibody or fragment thereof of clause 190, wherein antibody or fragment thereof as defined in any one of clauses 190 to 257, is an scFv, Fab, Fab', F(ab')₂, Fv, variable domain (e.g. VH or VL), diabody, minibody or full length antibody.

30 259. The anti-v δ 1 antibody or fragment thereof of clause 258, wherein the antibody or fragment thereof is an scFv or a full length antibody, such as IgG1.

35 260. The anti-v δ 1 antibody or fragment thereof of clause 259, wherein the antibody or fragment thereof comprises an amino acid sequence having at least 80% sequence identity with of any one of SEQ ID NOs: 111-122.

261. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 259 or clause 260, wherein the antibody or fragment thereof comprises an amino acid sequence of any one of SEQ ID NOs: 111-122.
- 5 262. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 111.
263. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 112.
- 10 264. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 116.
265. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 117.
- 15 266. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 118.
- 20 267. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 119.
268. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 260 or clause 261, wherein the antibody or fragment thereof comprises SEQ ID NO: 120.
- 25 269. The anti- $\nu\delta 1$ antibody or fragment thereof of any one of clauses 190 to 268, wherein the antibody or fragment thereof is human.
270. The anti- $\nu\delta 1$ antibody or fragment thereof of clause 190, wherein the anti- $\nu\delta 1$ antibody or fragment thereof with an EC50 value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.5 $\mu\text{g/ml}$; an EC50 value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.05 $\mu\text{g/ml}$; and/or an EC50 value for $\gamma\delta$ T cell killing upon binding which is less than 0.5 $\mu\text{g/ml}$.
- 30 271. An anti- $\nu\delta 1$ antibody or fragment thereof as defined in any one of clauses 190 to 270 for use in a method of modulating an immune response in a subject.
- 35

272. The anti- $\gamma\delta$ 1 antibody or fragment thereof of clause 271, wherein the subject has cancer, an infectious disease or an inflammatory disease.

273. The use of clause 271, wherein modulating an immune response in a subject comprises at least one selected from the group consisting of activating $\gamma\delta$ T cells, causing or increasing proliferation $\gamma\delta$ T cells, causing or increasing expansion of $\gamma\delta$ T cells, causing or increasing $\gamma\delta$ T cell degranulation, causing or increasing $\gamma\delta$ T cell killing activity, causing or increasing $\gamma\delta$ T cytotoxicity, causing or increasing $\gamma\delta$ T cell mobilization, increasing survival of $\gamma\delta$ T cells, and increasing resistance to exhaustion of $\gamma\delta$ T cells.

274. The use of any one of clauses 190 to 273, wherein diseased cells are killed while healthy cells are spared.

275. An isolated multi-specific antibody or fragment thereof that binds to at least two target antigens, wherein the first of the at least two target antigens is $\gamma\delta$ 1, and which comprises one or more of:

a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-25;

a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37 and SEQUENCES: A1-A12 (of Table 3); and/or

a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-61.

276. The isolated multi-specific antibody or fragment thereof as defined in clause 275, which comprises a VH region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 2-13, such as SEQ ID NOs: 8, 9, 10 or 11.

277. The isolated multi-specific antibody or fragment thereof as defined in clause 275 or clause 276, which comprises a VH region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 26-37, such as SEQ ID NOs: 32, 33, 34 or 35.

278. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 277, which comprises a VH region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 38-49, such as SEQ ID NOs: 44, 45, 46 or 47.

279. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 278, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 8, a CDR2 comprising a sequence of SEQ ID NO: 32, and a CDR1 comprising a sequence of SEQ ID NO: 44.

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280. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 278, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 9, a CDR2 comprising a sequence of SEQ ID NO: 33, and a CDR1 comprising a sequence of SEQ ID NO: 45.

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281. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 278, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 10, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR1 comprising a sequence of SEQ ID NO: 46.

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282. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 278, which comprises a VH region comprising a CDR3 comprising a sequence of SEQ ID NO: 11, a CDR2 comprising a sequence of SEQ ID NO: 35, and a CDR1 comprising a sequence of SEQ ID NO: 47.

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283. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 282, which comprises a VL region comprising a CDR3 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 14-25, such as SEQ ID NOs: 20, 21, 22 or 23.

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284. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 283, which comprises a VL region comprising a CDR2 comprising a sequence having at least 80% sequence identity with any one of SEQUENCES: A1-A12, such as SEQ ID NOs: A7, A8, A9 or A10.

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285. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 284, which comprises a VL region comprising a CDR1 comprising a sequence having at least 80% sequence identity with any one of SEQ ID NOs: 50-61, such as SEQ ID NOs: 56, 57, 58 or 59.

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286. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 284, which comprises a VL region comprising a CDR3 comprising a sequence

of SEQ ID NO: 20, a CDR2 comprising a sequence of SEQUENCE: A7, and a CDR1 comprising a sequence of SEQ ID NO: 56.

287. The isolated multi-specific antibody or fragment thereof as defined in any one of
5 clauses 275 to 284, which comprises a VL region comprising a CDR3 comprising a sequence
of SEQ ID NO: 21, a CDR2 comprising a sequence of SEQUENCE: A8, and a CDR1
comprising a sequence of SEQ ID NO: 57.

288. The isolated multi-specific antibody or fragment thereof as defined in any one of
10 clauses 275 to 284, which comprises a VL region comprising a CDR3 comprising a sequence
of SEQ ID NO: 22, a CDR2 comprising a sequence of SEQUENCE: A9, and a CDR1
comprising a sequence of SEQ ID NO: 58.

289. The isolated multi-specific antibody or fragment thereof as defined in any one of
15 clauses 275 to 284, which comprises a VL region comprising a CDR3 comprising a sequence
of SEQ ID NO: 23, a CDR2 comprising a sequence of SEQUENCE: A10, and a CDR1
comprising a sequence of SEQ ID NO: 59.

290. An isolated multi-specific antibody or fragment thereof which comprises a VH region
20 comprising CDR1, CDR2 and CDR3 sequences as defined in clause 279 and a VL region
comprising CDR1, CDR2 and CDR3 sequences as defined in clause 286.

291. An isolated multi-specific antibody or fragment thereof which comprises a VH region
comprising CDR1, CDR2 and CDR3 sequences as defined in clause 280 and a VL region
25 comprising CDR1, CDR2 and CDR3 sequences as defined in clause 287.

292. An isolated multi-specific antibody or fragment thereof which comprises a VH region
comprising CDR1, CDR2 and CDR3 sequences as defined in clause 281 and a VL region
comprising CDR1, CDR2 and CDR3 sequences as defined in clause 289.
30

293. An isolated multi-specific antibody or fragment thereof which comprises a VH region
comprising CDR1, CDR2 and CDR3 sequences as defined in clause 282 and a VL region
comprising CDR1, CDR2 and CDR3 sequences as defined in clause 289.

35 294. An isolated multi-specific antibody or fragment thereof, which comprises an amino acid
sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-85.

295. The isolated multi-specific antibody or fragment thereof as defined in clause 238, which comprises a VH region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62-73.
- 5 296. The isolated multi-specific antibody or fragment thereof as defined in clause 239 wherein the VH region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 62, 63, 64, 68, 69, 70 or 71.
297. The isolated multi-specific antibody or fragment thereof as defined in any one of
10 clauses 294 to 296, which comprises a VL region comprising an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74-85.
298. The isolated multi-specific antibody or fragment thereof as defined in clause 297,
15 wherein the VL region comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 74, 75, 76, 80, 81, 82 or 83.
299. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 62 and a VL region comprising an amino acid sequence of SEQ ID NO: 74.
20
300. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 63 and a VL region comprising an amino acid sequence of SEQ ID NO: 75.
- 25 301. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 64 and a VL region comprising an amino acid sequence of SEQ ID NO: 76.
302. The isolated multi-specific antibody or fragment thereof as defined in any one of
30 clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 68 and a VL region comprising an amino acid sequence of SEQ ID NO: 80.
303. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ
35 ID NO: 69 and a VL region comprising an amino acid sequence of SEQ ID NO: 81.

304. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 70 and a VL region comprising an amino acid sequence of SEQ ID NO: 82.
- 5 305. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 298, which comprises a VH region comprising an amino acid sequence of SEQ ID NO: 71 and a VL region comprising an amino acid sequence of SEQ ID NO: 83.
- 10 306. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 294 to 305, wherein the VH and VL region are joined by a linker, such as a polypeptide linker.
307. The isolated multi-specific antibody or fragment thereof as defined in clause 306, wherein the linker comprises a (Gly4Ser) n format, where $n = 1$ to 8
- 15 308. The isolated multi-specific antibody or fragment thereof as defined in clause 306 or clause 307, wherein the linker comprises a [(Gly4Ser) n (Gly3AlaSer) m] p linker, where n , m and $p = 1$ to 8.
- 20 309. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 306 to 307, wherein the linker comprises SEQ ID NO: 98.
310. The isolated multi-specific antibody or fragment thereof as defined in clause 309, wherein the linker consists of SEQ ID NO: 98.
- 25 311. An isolated multi-specific antibody or fragment thereof which comprises an amino acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 86-97.
312. The isolated multi-specific antibody or fragment thereof as defined in clause 311, which
- 30 comprises an amino acid sequence of any one of SEQ ID NOs: 86-97.
313. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or clause 311, which comprises SEQ ID NO: 86.
- 35 314. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or clause 311, which comprises SEQ ID NO: 87.

315. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or clause 311, which comprises SEQ ID NO: 88.
316. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or
5 clause 311, which comprises SEQ ID NO: 92.
317. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or clause 311, which comprises SEQ ID NO: 93.
- 10 318. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or clause 311, which comprises SEQ ID NO: 94.
319. The isolated multi-specific antibody or fragment thereof as defined in clause 283 or clause 311, which comprises SEQ ID NO: 95.
15
320. An isolated multi-specific antibody or fragment thereof, which binds to the same, or essentially the same, V δ 1 epitope as, or competes with, an antibody or fragment thereof as defined in any one of clauses 275 to 319.
- 20 321. A human, isolated anti-TCR delta variable 1, multi-specific antibody or fragment thereof, that binds to at least two target antigens, wherein the first of the at least two target antigens is V δ 1, and wherein the multi-specific antibody or fragment thereof which binds to an epitope of V δ 1 comprising one or more amino acid residues within amino acids 1-90 of SEQ ID NO: 1.
25
322. The human, isolated multi-specific antibody or fragment thereof as defined in clause 321, wherein the epitope comprises at least one of amino acid residues 3, 5, 9, 10, 12, 16, 17, 20, 37, 42, 50, 53, 59, 62, 64, 68, 69, 72 or 77 of SEQ ID NO: 1.
- 30 323. The human, isolated multi-specific antibody or fragment thereof as defined in clause 321 or clause 322, wherein the epitope comprises one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69, of SEQ ID NO: 1.
- 35 324. The human, isolated multi-specific antibody or fragment thereof as defined in clause 323, wherein the epitope consists of one or more amino acid residues within amino acid regions: 5-20 and 62-77; 50-64; 37-53 and 59-72; 59-77; or 3-17 and 62-69 of SEQ ID NO: 1.

325. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 321 to 324, wherein the epitope comprises one or more amino acid residues within amino acid regions 5-20 and 62-77 of SEQ ID NO: 1.

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326. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 321 to 324, wherein the epitope comprises one or more amino acid residues within amino acid region 50-64 of SEQ ID NO: 1.

10 327. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 321 to 324, wherein the epitope comprises one or more amino acid residues within amino acid regions 37-53 and 59-77 of SEQ ID NO: 1.

328. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 321 to 327, wherein the epitope is an activating epitope of a $\gamma\delta$ T cell.

329. The human, isolated multi-specific antibody or fragment thereof as defined in clause 328, wherein binding of the activating epitope: (i) downregulates the $\gamma\delta$ TCR; (ii) activates degranulation of the $\gamma\delta$ T cell; and/or (iii) activates $\gamma\delta$ T cell killing.

20

330. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 321 to 329, which only binds to an epitope in the V region of a V δ 1 chain of a $\gamma\delta$ TCR.

25 331. The human, isolated multi-specific antibody or fragment thereof as defined in any one of clauses 321 to 330, which does not bind to an epitope found in CDR3 of a V δ 1 chain of a $\gamma\delta$ TCR.

30 332. The human isolated multi-specific antibody or fragment thereof as defined in clause 331, which does not bind to an epitope within amino acid region 91-105 (CDR3) of SEQ ID NO: 1.

333. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 332, which binds a variable delta 1 (V δ 1) chain of a $\gamma\delta$ T cell receptor (TCR) with a binding affinity (KD) as measured by surface plasmon resonance of less than 1.5×10^{-7} M.

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334. The isolated multi-specific antibody or fragment thereof as defined in clause 333, wherein the K_D is less than 1.3×10^{-7} M or less, such as less than 1.0×10^{-7} M, in particular less than 5.0×10^{-8} M.

5 335. An isolated multi-specific antibody or fragment thereof with an EC_{50} value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.5 $\mu\text{g/ml}$.

336. An isolated multi-specific antibody or fragment thereof with an EC_{50} value for downregulation of a $\gamma\delta$ TCR upon binding which is less than 0.06 $\mu\text{g/ml}$.

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337. An isolated multi-specific antibody or fragment thereof with an EC_{50} value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.05 $\mu\text{g/ml}$.

338. An isolated multi-specific antibody or fragment thereof with an EC_{50} value for $\gamma\delta$ T cell degranulation upon binding which is less than 0.005 $\mu\text{g/ml}$, such as less than 0.002 $\mu\text{g/ml}$.

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339. The isolated multi-specific antibody or fragment thereof as defined in clause 337 or clause 338, wherein the $\gamma\delta$ T cell degranulation EC_{50} value is measured by detecting CD107a expression.

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340. An isolated multi-specific antibody or fragment thereof with an EC_{50} value for $\gamma\delta$ T cell killing upon binding which is less than 0.5 $\mu\text{g/ml}$.

341. An isolated multi-specific antibody or fragment thereof with an EC_{50} value for $\gamma\delta$ T cell killing upon binding which is less than 0.055 $\mu\text{g/ml}$, such as less than 0.020 $\mu\text{g/ml}$.

25

342. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 335 to 341, wherein the EC_{50} value is measured using flow cytometry.

30 343. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 342, which is an scFv, Fab, Fab', F(ab')₂, Fv, variable domain (e.g. VH or VL), diabody, minibody or full length antibody.

34 344. The isolated multi-specific antibody or fragment thereof as defined in clause 343, which is an scFv or a full length antibody, such as IgG1.

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345. The isolated multi-specific antibody as defined in clause 344, which comprises an amino acid sequence having at least 80% sequence identity with of any one of SEQ ID NOs: 111-122.

- 5 346. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises an amino acid sequence of any one of SEQ ID NOs: 111-122.

347. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 111.

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348. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 112.

- 15 349. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 116.

350. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 117.

- 20 351. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 118.

352. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 119.

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353. The isolated multi-specific antibody as defined in clause 344 or clause 345, which comprises SEQ ID NO: 120.

- 30 354. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 353, which is human.

- 35 355. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 375 to 354, wherein the second of the at least two target antigens is one selected from the group consisting of CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3, CD3d, CD3e, CD3g, CD4, CD5, CD6, CD7, CD8, CD8a, CD8b, CD9, CD10, CD11a, CD11b, CD11c, CD11d, CD13, CD14, CD15, CD16, CD16a, CD16b, CD17, CD18, CD19, CD20, CD21, CD22, CD23, CD24, CD25, CD26, CD27, CD28, CD29, CD30, CD31, CD32A, CD32B, CD33, CD34,

CD35, CD36, CD37, CD38, CD39, CD40, CD41, CD42, CD42a, CD42b, CD42c, CD42d, CD43, CD44, CD45, CD46, CD47, CD48, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD51, CD52, CD53, CD54, CD55, CD56, CD57, CD58, CD59, CD60a, CD60b, CD60c, CD61, CD62E, CD62L, CD62P, CD63, CD64a, CD65, CD65s, CD66a, CD66b, CD66c, 5 CD66d, CD66e, CD66f, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD77, CD79A, CD79B, CD80, CD81, CD82, CD83, CD84, CD85A, CD85B, CD85C, CD85D, CD85F, CD85G, CD85H, CD85I, CD85J, CD85K, CD85M, CD86, CD87, CD88, CD89, CD90, CD91, CD92, CD93, CD94, CD95, CD96, CD97, CD98, CD99, CD100, CD101, CD102, CD103, CD104, CD105, CD106, CD107, CD107a, CD107b, CD108, CD109, CD110, CD111, 10 CD112, CD113, CD114, CD115, CD116, CD117, CD118, CD119, CD120, CD120a, CD120b, CD121a, CD121b, CD122, CD123, CD124, CD125, CD126, CD127, CD129, CD130, CD131, CD132, CD133, CD134, CD135, CD136, CD137, CD138, CD139, CD140A, CD140B, CD141, CD142, CD143, CD144, CDw145, CD146, CD147, CD148, CD150, CD151, CD152, CD153, CD154, CD155, CD156, CD156a, CD156b, CD156c, CD157, CD158, CD158A, CD158B1, 15 CD158B2, CD158C, CD158D, CD158E1, CD158E2, CD158F1, CD158F2, CD158G, CD158H, CD158I, CD158J, CD158K, CD159a, CD159c, CD160, CD161, CD162, CD163, CD164, CD165, CD166, CD167a, CD167b, CD168, CD169, CD170, CD171, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD177, CD178, CD179a, CD179b, CD180, CD181, CD182, CD183, CD184, CD185, CD186, CD187, CD188, CD189, 20 CD190, CD191, CD192, CD193, CD194, CD195, CD196, CD197, CDw198, CDw199, CD200, CD201, CD202b, CD203c, CD204, CD205, CD206, CD207, CD208, CD209, CD210, CDw210a, CDw210b, CD211, CD212, CD213a1, CD213a2, CD214, CD215, CD216, CD217, CD218a, CD218b, CD219, CD220, CD221, CD222, CD223, CD224, CD225, CD226, CD227, CD228, CD229, CD230, CD231, CD232, CD233, CD234, CD235a, CD235b, CD236, CD237, 25 CD238, CD239, CD240CE, CD240D, CD241, CD242, CD243, CD244, CD245[17], CD246, CD247, CD248, CD249, CD250, CD251, CD252, CD253, CD254, CD255, CD256, CD257, CD258, CD259, CD260, CD261, CD262, CD263, CD264, CD265, CD266, CD267, CD268, CD269, CD270, CD271, CD272, CD273, CD274, CD275, CD276, CD277, CD278, CD279, CD280, CD281, CD282, CD283, CD284, CD285, CD286, CD287, CD288, CD289, CD290, 30 CD291, CD292, CDw293, CD294, CD295, CD296, CD297, CD298, CD299, CD300A, CD300C, CD301, CD302, CD303, CD304, CD305, CD306, CD307, CD307a, CD307b, CD307c, CD307d, CD307e, CD308, CD309, CD310, CD311, CD312, CD313, CD314, CD315, CD316, CD317, CD318, CD319, CD320, CD321, CD322, CD323, CD324, CD325, CD326, CD327, CD328, CD329, CD330, CD331, CD332, CD333, CD334, CD335, CD336, CD337, 35 CD338, CD339, CD340, CD344, CD349, CD351, CD352, CD353, CD354, CD355, CD357, CD358, CD360, CD361, CD362, CD363, CD364, CD365, CD366, CD367, CD368, CD369, CD370, and CD371.

356. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 375 to 354, wherein the second of the at least two target antigens is one selected from the group consisting of AFP, AKAP-4, ALK, alphafetoprotein, Androgen receptor, B7H3, BAGE, BCA225, BCAA, Bcr-abl, beta-Catenin, beta-HCG, beta-human chorionic gonadotropin, BORIS, BTAA, CA 125, CA 15-3, CA 195, CA 19-9, CA 242, CA 27.29, CA 72-4, CA-50, CAM 17.1, CAM43, Carbonic anhydrase IX, carcinoembryonic antigen, CD22, CD33/IL3Ra, CD68P1, CDK4, CEA, chondroitin sulfate proteoglycan 4 (CSPG4), c-Met, CO-029, CSPG4, Cyclin B1, cyclophilin C-associated protein, CYP1B1, E2A-PRL, EGFR, EGFRvIII, ELF2M, EpCAM, EphA2, EphrinB2, Epstein Barr virus antigens EBVA, ERG (TMPRSS2ETS fusion gene), ETV6-AML, FAP, FGF-5, Fos-related antigen 1, Fucosyl GM1, G250, Ga733\EpCAM, GAGE-1, GAGE-2, GD2, GD3, glioma-associated antigen, GloboH, Glycolipid F77, GM3, GP 100, GP 100 (Pmel 17), H4-RET, HER-2/neu, HER-2/Neu/ErbB-2, high-molecular-weight melanoma-associated antigen (HMW-MAA), HPV E6, HPV E7, hTERT, HTgp-175, human telomerase reverse transcriptase, Idiotypic, IGF-I receptor, IGF-II, IGH-IGK, insulin growth factor (IGF)-I, intestinal carboxyl esterase, K-ras, LAGE-1a, LCK, lectin-reactive AFP, Legumain, LMP2, M344, MA-50, Mac-2 binding protein, MAD-CT-1, MAD-CT-2, MAGE, MAGE A1, MAGE A3, MAGE-1, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MART-1, MART-1/MelanA, M-CSF, melanoma-associated chondroitin sulfate proteoglycan (MCSP), Mesothelin, MG7-Ag, ML-IAP, MN-CA IX, MOV18, MUC1, Mum-1, hsp70-2, MYCN, MYL-RAR, NA17, NB/70K, neuron-glia antigen 2 (NG2), neutrophil elastase, nm-23H1, NuMa, NY-BR-1, NY-CO-1, NY-ESO, NY-ESO-1, NY-ESO-1, OY-TES1, p15, p16, p180erbB3, p185erbB2, p53, p53 mutant, Page4, PAX3, PAX5, PDGFR-beta, PLAC1, Polysialic Acid, prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen, prostatic acid phosphatase (PAP), Proteinase3 (PR1), PSA, PSCA, PSMA, RAGE-1, Ras, Ras-mutant, RCAS1, RGS5, RhoC, ROR1, RU1, RU2 (AS), SART3, SDCCAG16, sLe(a), Sperm protein 17, SSX2, STn, Survivin, TA-90, TAAL6, a TAG-72, telomerase, thyroglobulin, Tie 2, TIGIT, TLP, Tn, TPS, TRP-1, TRP-2, TRP-2, TSP-180, Tyrosinase, VEGFR2, VISTA, WT1, XAGE 1, 43-9F, 5T4, and 791Tgp72.

30

357. The isolated multi-specific antibody or fragment thereof as defined in any one of clauses 375 to 354, wherein the multi-specific antibody format is one selected from the groups consisting of CrossMab, DAF (two-in-one), DAF (four-in-one), DutaMab, DT-IgG, Knobs-in-holes (KIH), Knobs-in-holes (common light chain), Charge pair, Fab-arm exchange, SEEDbody, Triomab, LUZ-Y, Fcab, $\kappa\lambda$ -body, orthogonal Fab, DVD-IgG, IgG(H)-scFv, scFv-(H)IgG, IgG(L)-scFv, scFv-(L)IgG, IgG(L,H)-Fv, IgG(H)-V, V(H)-IgG, IgG(L)-V, V(L)-IgG, KIH IgG-scFab, 2scFv-IgG, IgG-2scFv, scFv4-Ig, Zyboby, DVI-IgG(four-in-one), Nanobody,

35

Nanoby-HAS, BiTE, Diabody, DART, TandAb, scDiabody, scDiabody-CH3, Diabody-CH3, Triple Body, Miniantibody, Minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv, scFV-CH-CL-scFv, F(ab')₂, F(ab')₂-scFv₂, scFv-KIH, Fab-scFv-Fc, Tetravalent HCAb, scDiabody-Fc, Diabody-Fc, Tandem scFv-Fc, Intrabody, Dock and Lock, ImmTAC, HSAbody, scDiabody-HAS, Tandem scFv-Toxin, IgG-IgG, ov-X-Body, duobody, mab2 and scFv1-PEG-scFv₂.

358. A polynucleotide sequence encoding the multi-specific antibody or fragment thereof as defined in any one of clauses 375 to 357.

10 359. A polynucleotide sequence encoding the multi-specific antibody or fragment thereof comprising a sequence having at least 70% sequence identity with SEQ ID NO: 99-110.

360. A polynucleotide sequence encoding the multi-specific antibody or fragment thereof comprising a sequence of SEQ ID NO: 99-110.

15

361. An expression vector encoding the multi-specific antibody or fragment thereof comprising the polynucleotide sequence as defined in any one of clauses 358 to 361.

20 362. An expression vector encoding the multi-specific antibody or fragment thereof comprising the VH region of SEQ ID NO: 99-110.

363. An expression vector encoding the multi-specific antibody or fragment thereof comprising the VL region of SEQ ID NO: 99-110.

25 364. An expression vector encoding the multi-specific antibody or fragment thereof comprising the VH region of clause 117 and the VL region of clause 307.

30 365. A cell comprising the polynucleotide sequence encoding the multi-specific antibody or fragment thereof as defined in any one of clauses 358 to 360 or the expression vector as defined in any one of clauses 361 to 364.

366. A cell comprising a first expression vector encoding the multi-specific antibody or fragment thereof as defined in clause 362 and a second expression vector encoding the multi-specific antibody or fragment thereof as defined in clause 363.

35

367. A cell comprising the expression vector encoding the multi-specific antibody or fragment thereof as defined in clause 364.

368. The cell as defined in any one of clauses 365 to 367, wherein the polynucleotide or expression vector encoding the multi-specific antibody or fragment thereof encodes a membrane anchor or transmembrane domain fused to the antibody or fragment thereof,
5 wherein the antibody or fragment thereof is presented on an extracellular surface of the cell.

369. A composition comprising the multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 357.

10 370. A pharmaceutical composition comprising the multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 357, together with a pharmaceutically acceptable diluent or carrier.

371. The isolated multi-specific antibody or fragment thereof as defined in any one of
15 clauses 275 to 357 or the pharmaceutical composition as defined in clause 314, for use as a medicament.

372. The isolated multi-specific antibody or fragment thereof or the pharmaceutical composition as defined in clause 371 for use in the treatment of cancer, an infectious disease
20 or an inflammatory disease.

373. An isolated multi-specific antibody or fragment thereof as defined in any one of clauses 275 to 357 or the pharmaceutical composition as defined in clause 314 for use in the treatment of a cancer, an infectious disease or an inflammatory disease.
25

374. An isolated antigen comprising an amino acid sequence having at least 80% sequence identity with SEQ ID NO: 123 for use in generating a multi-specific antibody or fragment thereof.

30 375. A method of generating a multi-specific antibody or fragment thereof comprising:
(i) designing a series of antigens comprising a TCR delta variable 1 (TRDV1) amino acid sequence wherein the CDR3 sequence of the TRDV1 is the same for all antigens in the series;
(ii) exposing a first antigen designed in step (i) to an antibody library;
(iii) isolating the antibodies or fragments thereof which bind to the antigen;
35 (iv) exposing the isolated antibodies or fragments thereof to a second antigen designed in step (i); and

(v) isolating the antibodies or fragments thereof which bind to both the first and second antigen.

376. The method as defined in clause 375, which further comprises exposing the isolated
5 antibodies or fragments thereof to a second series of antigens comprising a $\gamma\delta$ TCR with a different delta variable chain, such as TCR delta variable 2 (TRDV2) or TCR delta variable 3 (TRDV3), and then deselecting the antibodies or fragments thereof which also bind to the second series of antigens.

10 377. The method as defined in clause 375 or clause 376, wherein the first and/or second series of antigens are presented as a leucine zipper and/or Fc fusion.

378. The method as defined in any one of clauses 375 to 377, wherein the series of antigens
15 are in a heterodimeric and/or homodimeric format.

379. An antibody obtained by the method as defined in any one of clauses 375 to 378.

Other features and advantages of the present invention will be apparent from the description
provided herein. It should be understood, however, that the description and the specific
20 examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications will become apparent to those skilled in the art. The invention will now be described using the following, non-limiting examples:

25 EXAMPLES

EXAMPLE 1. Materials and Methods

Human Antibody Discovery

Human phage display was employed to generate the human anti-human variable V δ 1+
30 domain antibodies as described herein. The library was constructed as described in Schofield et al (*Genome biology* 2007, 8(11): R254) and comprised a single chain fragment variable (scFv) displaying library of ~40 billion human clones. This library was screened using antigens, methods, selections, deselection, screening, and characterization strategies as described herein.

35

Antigen preparation

The design of the soluble $\gamma\delta$ TCR heterodimers comprising the TCR α and TCR β constant regions used in the below Examples were generated according to Xu *et al.* (2011) *PNAS* 108: 2414-2419. V γ or V δ domains were fused in-frame to a TCR α or TCR β constant region lacking the transmembrane domain, followed by a leucine zipper sequence or an Fc sequence, and a histidine tag/linker.

The expression construct was transiently transfected in mammalian EXPI HEK293 suspension cells (either as single or co-transfections for heterodimer). Secreted recombinant proteins were recovered and purified from culture supernatant by affinity chromatography. To ensure good recovery of monomer antigen, samples were further purified using preparative size exclusion chromatography (SEC). Purified antigens were analysed for purity by SDS-PAGE and aggregation state by analytical SEC.

Antigen functional validation

The specificity of the antigens containing delta variable 1 (V δ 1) chain was confirmed in DELFIA immunoassay (Perkin Elmer) and in flow-based assay in competition with $\gamma\delta$ T cells using REA173-Miltenyi Biotec anti-V δ 1 antibody.

Dissociation-enhanced lanthanide fluorescence immunoassay (DELFLIA)

For the confirmation of antigen's specificity, DELFLIA immunoassay was performed with the antigen directly coated to the plate (3 μ g/mL of antigen in 50 μ L PBS at 4 °C overnight (Nunc #437111) and serial dilution of primary antibodies starting at 300nM. For detection DELFLIA Eu-N1 Anti-Human IgG (Perkin Elmer # 1244-330) was used as secondary antibody at 1/500 dilution in 50 μ L of 3 % of MPBS (PBS + 3 % (w/v) skimmed milk powder). Development was with 50 μ L of DELFLIA enhancement solution (Perkin Elmer #4001-0010).

Affinity ranking of antibody of interest were performed using DELFLIA immunoassay in which antibodies were captured via protein G coated on the plate and soluble biotinylated L1 (DV1-GV4) antigen was added at 5nM in 50 μ L (3MPBS). For detection 50 μ L of streptavidin-Eu (1:500 in assay buffer, Perkin Elmer) was used and signal was developed with DELFLIA enhancement solution. D1.3 hIgG1 (described in England *et al.* (1999) *J. Immunol.* 162: 2129-2136) was used as a negative control.

Phage display selection outputs were subcloned into the scFv expression vector pSANG10 (Martin *et al.* (2006) *BMC Biotechnol.* 6: 46). Soluble scFv were expressed and screened for binding in DELFLIA on directly immobilised targets. Hits were defined as a DELFLIA signal above 3000 fluorescence units.

Antibody preparation

- Selected scFvs were subcloned into IgG1 frameworks using commercially available plasmids. expi293F suspension cells were transfected with said plasmids for antibody expression. For
- 5 convenience, unless otherwise noted, the antibodies characterised in these Examples refer to IgG1 formatted antibodies selected from phage display as scFv. However, the antibodies of the invention may be in any antibody format as previously discussed.

Antibody purification

- 10 IgG antibodies were batch purified from supernatants using protein A chromatography. Concentrated protein A eluates were then purified using Size Exclusion Chromatography (SEC). Quality of purified IgG was analysed using ELISA, SDS-PAGE and SEC-HPLC.

$\gamma\delta$ T cell preparation

- 15 Populations of enriched $\gamma\delta$ T cells were prepared according to the methods described in WO2016/198480 (i.e. blood-derived $\gamma\delta$ T cells) or WO2020/095059 (i.e. skin-derived $\gamma\delta$ T cells). Briefly, for blood-derived $\gamma\delta$ T cells PBMCs were obtained from blood and subjected to magnetic depletion of $\alpha\beta$ T cells. The $\alpha\beta$ -depleted PBMCs were then cultured in CTS OpTmiser media (ThermoFisher) in the presence of OKT-3 (or respective anti-V δ 1 antibody),
- 20 IL-4, IFN- γ , IL-21 and IL-1 β for 7 days. At day 7 of culture, the media was supplemented with OKT-3 (or respective anti-V δ 1 antibody), IL-21 and IL-15 for a further 4 days. At day 11 of culture, the media was supplemented with OKT-3 (or respective anti-V δ 1 antibody) and IL-15 for a further 3 days. At day 14 of culture, half of the media was replaced with fresh complete OpTmiser and supplemented with OKT-3 (or respective anti-V δ 1 antibody), IL-15 and IFN- γ .
- 25 From day 17 of culture onwards, the culture was supplemented with OKT-3 (or respective anti-V δ 1 antibody) and IL-15 every 3 to 4 days; half of the media was replaced with fresh media every 7 days.

- For skin-derived $\gamma\delta$ T cells, skin samples are prepared by removing subcutaneous fat and a
- 30 3mm biopsy punch is used to make multiple punches. Punches are placed on carbon matrix grids and placed in the well of a G-REX6 (Wilson Wolf). Each well is filled with complete isolation medium containing AIM-V media (Gibco, Life Technologies), CTS Immune Serum Replacement (Life Technologies), IL-2 and IL-15. For the first 7 days of culture, complete isolation medium containing Amphotericin B (Life Technologies) was used ("AMP"). Media
- 35 was changed every 7 days by gently aspirating the upper media and replacing with 2X complete isolation medium (without AMP), trying not to disturb the cells at the bottom of the plate or bioreactor. Beyond three weeks in culture, the resulting egressed cells are then

passaged into fresh tissue culture vessels and fresh media (e.g. AIM-V media or TexMAX media (Miltenyi)) plus recombinant IL-2, IL-4, IL-15 and IL-21 before harvest. Optionally, $\alpha\beta$ T cells also present within the culture are then removed with aid of $\alpha\beta$ T cell depletion kits and associated protocols, such as those provided by Miltenyi. For further reference see

5 WO2020/095059.

$\gamma\delta$ T cell binding assay

The binding of antibodies to $\gamma\delta$ T cells was tested by incubating a fixed concentration of purified antibodies with 250000 $\gamma\delta$ T cells. This incubation was performed under blocking

10 conditions to prevent unspecific binding of antibodies via the Fc receptor. Detection was performed by addition of a secondary, fluorescent dye-conjugated antibody against human IgG1. For negative controls, cells were prepared with a) an isotype antibody only (recombinant human IgG), b) the fluorescent dye-conjugated anti-human IgG antibody only and c) a combination of a) and b). A control well of completely unstained cells was also prepared and

15 analysed. As positive controls, a purified murine monoclonal IgG2 anti-human CD3 antibody and a purified murine monoclonal IgG1 anti-human TCR V δ 1 antibody were used in two different concentrations and stained with a fluorescent dye-conjugated goat anti-mouse secondary antibody. The assay was accepted if the lower concentration positive controls' mean fluorescence intensity in the FITC channel was at least tenfold as high as the highest

20 negative control.

SPR Analysis

A MASS-2 instrument with an amine high capacity chip (both from Sierra Sensors, Germany) was used to perform SPR analysis. 15 nM IgG were captured via protein G to an amine high

25 capacity chip (100 nM for TS8.2). L1 (DV1-GV4) antigen was flown over the cell at a 1:2 dilution series from 2000 nM to 15.625 nM with the following parameters: 180 s association, 600 s dissociation, flowrate 30 μ L/min, running buffer PBS + 0.02 % Tween 20. All experiments were performed at room temperature on MASS-2 instrument. Steady state fitting was determined according to Langmuir 1:1 binding using software Sierra Analyzer 3.2.

30

Comparator antibodies

Antibodies of the invention were compared to commercially available antibodies in test assays as described.

Antibody	Source	Catalogue No.
Ultra-LEAF™ Purified anti-human CD3 Antibody (OKT3), functional	Biolegend	317326

Ultra-LEAF™ Purified anti-human IgG2a Antibody (isotype control for OKT3)	Biolegend	400264
Human TCR Vδ1 purified mAb (functional TS8.2)	ThermoFisher	TCR1730
Ultra-LEAF™ Purified Mouse IgG1, κ Isotype Ctrl Antibody for TS8.2	Biolegend	400166
Anti-human CD107a BV421 (clone H4A3)	BD Biosciences	562623
BV421 Mouse IgG1, κ Isotype Control Clone X40 (RUO)	BD Biosciences	562438
Anti-TCR Vδ1-PE-Vio770, human (flow, discontinued)	Miltenyi	130-100-540
Cetuximab	In-house	-
D1.3 HEL (anti-chicken lysozyme; mouse VH-VL + Human IgG1 Fc).	In-house	-
Anti-RSV (aka Motavizumab)	In-house	-

γδ TCR downregulation and degranulation assay

THP-1 (TIB-202™, ATCC) target cells loaded or not with test antibodies were labelled with CellTracker™ Orange CMTMR (ThermoFisher, C2927) and incubated with γδ T cells at 2:1 ratio in the presence of CD107a antibody (Anti-human CD107a BV421 (clone H4A3) BD Biosciences 562623). After 2 hours of incubation, the surface expression of γδ TCR (to measure TCR downregulation) and expression of CD107a (to measure degranulation) on γδ T cells was evaluated using flow cytometry.

10 Killing assay (e.g. for Figure 6)

Gamma delta T cell killing activity and effect of test antibodies on the killing activity of γδ T cells was accessed by flow cytometry. After 4 hours of *in vitro* co-culture at 20:1 ratio of γδ T cells and CellTracker™ Orange CMTMR (ThermoFisher, C2927) labelled THP-1 cells (loaded or not with the antibody) were stained with Viability Dye eFluor™ 520 (ThermoFisher, 520 65-0867-14) to distinguish between live and dead target THP-1 cells. During sample acquisition, target cells were gated on the CellTracker™ Orange CMTMR positivity and examined for cell death based on the uptake of Viability Dye. CMTMR and eFluor™ 520 double positive cells were recognized as the dead target cells. The killing activity of γδ T cells was presented as a % of the dead target cells.

Epitope mapping

All protein samples (antigen L1 (DV1-GV4) and antibodies 1245_P01_E07, 1245_P02_G04, 1252_P01_C08, 1251_P02_C05 and 1141_P01_E01) used for epitope mapping were
 5 analyzed for protein integrity and aggregation level using a high-mass MALDI.

In order to determine the epitope of L1(DV1-GV4)/1245_P01_E07, L1(DV1-GV4)/1245_P02_G04, L1(DV1-GV4)/1252_P01_C08, L1(DV1-GV4)/1251_P02_C05, and L1(DV1-GV4)/1141_P01_E01 complexes with high resolution, the protein complexes were
 10 incubated with deuterated cross-linkers and subjected to multi-enzymatic proteolysis using trypsin, chymotrypsin, Asp-N, elastase and thermolysin. After enrichment of the cross-linked peptides, the samples were analyzed by high resolution mass spectrometry (nLC-LTQ-Orbitrap MS) and the data generated were analyzed using XQuest and Stavrox software.

15 SYTOX-flow killing assay

The SYTOX assay allows the quantification of T cell mediated cytolysis of target cells using flow cytometry. Dead/dying cells are detected by a dead cell stain (SYTOX® AADvanced™, Life Technologies, S10274) which only penetrates into cells with compromised plasma membranes but cannot cross intact cell membranes of healthy cells. NALM-6 target cells
 20 were labelled with CTV dye (Cell Trace Violet™, Life Technologies, C34557) and were thus distinguishable from the unlabelled effector T cells. Dead/dying target cells are identified through double staining of the dead cell dye and the cell trace dye.

After 16 hours in vitro co-culture of effector and CTV labelled target cells at indicated Effector-to-Target ratios (E:T, 1:1 or 10:1) the cells were stained with SYTOX® AADvanced™ and
 25 acquired on a FACSLyric™ (BD). The killing results are presented as % target cell reduction which is calculated by taking into account the number of live target cells (sample counts) in the test samples over the live target cells in the control wells without added effector cells (maximum counts):

30

$$\% \text{ target reduction} = 100 - ((\text{sample counts} / \text{maximum counts}) \times 100)$$

EXAMPLE 2. Antigen design

Gamma delta (γδ) T cells are polyclonal with CDR3 polyclonality. In order to avoid a situation
 35 where generated antibodies would be selected against the CDR3 sequence (as the CDR3 sequence will differ from TCR clone to TCR clone), the antigen design involved maintaining a consistent CDR3 in different formats. This design aimed to generate antibodies recognising a

sequence within the variable domain, which is germline encoded and therefore the same in all clones, thus providing antibodies which recognise a wider subset of $\gamma\delta$ T cells.

Another important aspect of the antigen preparation process was to design antigens which are suitable for expression as a protein. The $\gamma\delta$ TCR is a complex protein involving a heterodimer with inter-chain and intra-chain disulphide bonds. A leucine zipper (LZ) format and Fc format were used to generate soluble TCR antigens to be used in the phage display selections. Both the LZ and Fc formats expressed well and successfully displayed the TCR (particularly heterodimeric TCRs, e.g. V δ 1V γ 4).

10

It was found that the CDR3 sequence from a public database entry for the $\gamma\delta$ TCR expressed well as proteins (RCSB Protein Data Bank entries: 3OMZ). This was therefore selected for antigen preparation.

Antigens containing the delta variable 1 chain were expressed in LZ formats as a heterodimer (*i.e.* in combination with different gamma variable chains – “L1”, “L2”, “L3”) and in Fc format either as a heterodimer (“F1”, “F2”, “F3”) or as a homodimer (*i.e.* in combination with another delta variable 1 chain – “Fc1/1”). All delta variable 1 chains of the antigens contained the 3OMZ CDR3. Another series of $\gamma\delta$ TCR antigens using similar formats were designed containing different delta variable chains (such as delta variable 2 and delta variable 3) and used to deselect antibodies with non-specific or off target binding (“L4”, “F9”, “Fc4/4”, “Fc8/8”). These antigens were also designed to include the 3OMZ CDR3 to ensure that antibodies binding in the CDR3 region were also deselected.

Antigen functional validation was performed to confirm that the designed antigens would be suitable to generate anti-TRDV1 (TCR delta variable 1) antibodies. Detection was seen only with antigens containing the δ 1 domain (**Figure 1**).

EXAMPLE 3. Phage Display

Phage display selections were performed against libraries of human scFvs using either heterodimeric LZ TCR format in round 1 and 2, with deselections on heterodimeric LZ TCR in both rounds. Or round 1 was performed using homodimeric Fc fusion TCR with deselection on human IgG1 Fc followed by round 2 on heterodimeric LZ TCR with deselection on heterodimeric LZ TCR (see **Table 2**).

35

Table 2. Overview phage display selections

Target	Round 1 selection	Round 1 deselection	Round 2 selection	Round 2 deselection
DV1	bt-L1 (DV1-GV4)	L4 (DV2-GV4)	bt-L3 (DV1-GV8)	L4 (DV2-GV4)
DV1	bt-Fc1/1 (DV1-DV1)	Fc	bt-L1 (DV1-GV4)	L4 (DV2-GV4)

bt = biotin.

Selections were performed in solution phase using 100 nM biotinylated proteins. Deselections
5 were performed using 1 μ M non-biotinylated proteins.

Success of the phage display selections was analysed by polyclonal phage ELISA (DELFA).
All DV1 selection outputs showed the desired binding to the targets Fc 1/1, L1, L2, L3, F1 and
F3. Varying degrees of binding to non-targets L4, F9, Fc 4/4, Fc 8/8 and Fc were detected
10 (see **Figure 2A and B**).

EXAMPLE 4. Antibody selection

Hits obtained in Example 3 were sequenced (using standard methods known in the art). 130
unique clones were identified, which showed a unique combination of VH and VL CDR3. Of
15 these 130 unique clones, 125 showed a unique VH CDR3 and 109 showed a unique VL CDR3.

Unique clones were re-arrayed and specificity was analysed by ELISA (DELFA). A panel of
94 unique human scFv binders which bind TRDV1 (L1, L2, L3, F1, F2, F3) but not TRDV2
(L4), were identified from the selections.

20

Affinity ranking of the selected binders was included to aid the choice of clones going forward.
A large number of binders showed affinities in the nanomolar range, reacting with 25 to 100
nM biotinylated antigen. A handful of binders showed a strong reaction with 5 nM antigen,
indicating possible single digit nanomolar affinities. Some binders showed no reaction with
25 100 nM antigen, indicating affinities in the micromolar range.

For the selection of clones to proceed with to IgG conversion, the aim was to include as many
germline lineages and as many different CDR3s as possible. Further, sequence liabilities like
glycosylation, integrin binding sites, CD11c/CD18 binding sites, unpaired cysteines were
30 avoided. In addition, a variety of affinities was included.

Selected clones were screened for binding to natural, cell-surface expressed $\gamma\delta$ TCR using skin derived $\gamma\delta$ T cells obtained from different donors. The clones chosen to be converted to IgG are shown in **Table 3**.

GDT-P2851PCT

145

Table 3. DV1 binders for IgG conversion

Clone ID	Heavy CDR1	SEQ ID NO.	Heavy CDR2	SEQ ID NO.	Heavy CDR3	SEQ ID NO.	Light CDR1	SEQ ID NO.	Light CDR2	SEQ ID NO.	Light CDR3	SEQ ID NO.	100 nM L1
1245_P01_E07	GFTFSDYY	38	ISSSGSTI	26	VDYADAFDI	2	QSIGTY	50	VAS	A1	QQSYSTLLT	14	162591
1252_P01_C08	GFTVSSNY	39	IYSGGST	27	PIELGAFDI	3	NIGSQS	51	YDS	A2	QVWDSSSDHVV	15	1977
1245_P02_G04	GDSVSSKSAA	40	TYRRSKWST	28	TWSGYVDV	4	QDINDW	52	DAS	A3	QQSYSTPQVT	16	5896
1245_P01_B07	GFTFSDYY	41	ISSSGSTI	29	ENYLNAFDI	5	QSLSNY	53	AAS	A4	QQSYSTPLT	17	64271
1251_P02_C05	GFTFSSYA	42	ISGGGGTT	30	DSGVAFDI	6	QNIPTW	54	DAS	A5	QQFKRYPPT	18	65269
1141_P01_E01	GYSTSYW	43	IYPGDSST	31	HQVDTRTADY	7	RSDVGGYNY	55	EVS	A6	SSYTSTSTLV	19	136780
1139_P01_E04	GDSVSSNSAA	44	TYRRSKWYN	32	SWNDAFDI	8	QSISTW	56	DAS	A7	QQSYSTPLT	20	23786
1245_P02_F07	GDSVSSNSAA	45	TYRRSKWYN	33	DYYYSMDV	9	QSISSW	57	DAS	A8	QQSHSHPPT	21	10450
1245_P01_G06	GFTFSDYY	46	ISSSGSTI	34	HSWDAFDV	10	QSISSY	58	AAS	A9	QQSYSTPDT	22	22474
1245_P01_G09	GDSVSSNSAA	47	TYGSKWYN	35	DYYYSMDV	11	QSISTW	59	DAS	A10	QQSYSTPVT	23	18430
1138_P01_B09	GFTFSDYY	48	ISSSGSTI	36	HSWDAFDI	12	QDISNY	60	DAS	A11	QQSYSTPLT	24	29193
1251_P02_G10	GFTFSDYY	49	ISSSGSTI	37	HSWDAFDI	13	QSISSH	61	AAS	A12	QQSYSTLLT	25	17053

EXAMPLE 5: Antibody SPR analysis

Prepared IgG antibodies were passed through a $\gamma\delta$ cell binding assay, and 5 were selected for further functional and biophysical characterization. SPR analysis was performed to determine the equilibrium dissociation constants (K_D). Sensorgrams of the interaction of the tested antibody with the analyte, along with steady state fits (if available), are presented in **Figure 3**. No binding was detected for TS8.2 with 80 RU of IgG captured on the chip. Results are summarised in **Table 4**.

Table 4. Results of IgG capture

Analyte	Clone ID	K_D (nM)	K_D (M)
L1 (DV1-GV4)	1245_P01_E07	12.4	1.24e-08
L1 (DV1-GV4)	1252_P01_C08	100	1.00e-07
L1 (DV1-GV4)	1245_P02_G04	126	1.26e-07
L1 (DV1-GV4)	1245_P01_B07	341	3.41e-07
L1 (DV1-GV4)	1251_P02_C05	1967*	1.97e-06
L1 (DV1-GV4)	1139_P01_E04	251	2.51e-07
L1 (DV1-GV4)	1245_P02_F07	193	1.93e-07
L1 (DV1-GV4)	1245_P01_G06	264	2.64e-07
L1 (DV1-GV4)	1245_P01_G09	208	2.08e-07
L1 (DV1-GV4)	1138_P01_B09	290	2.90e-07
L1 (DV1-GV4)	1251_P02_G10	829	8.29e-07
L1 (DV1-GV4)	TS8.2 (commercial anti-V δ 1 antibody)	44	4.40e-08

10 *Binding of 1252_P02_C05 did not reach saturation, therefore data was extrapolated

EXAMPLE 6: TCR engagement assay

The inventors designed several assays to be used for functional characterization of the selected antibodies. The first assay assessed $\gamma\delta$ TCR engagement by measuring downregulation of the $\gamma\delta$ TCR upon antibody binding. Selected antibodies were tested against commercial anti-CD3 and anti-V δ 1 antibodies which were used as positive controls or against 1252_P01_C08 as a positive control (for 1139_P01_E04, 1245_P02_F07, 1245_P01_G06 and 1245_P01_G09). Commercial anti-p $\alpha\gamma\delta$ was used as a negative control because it is a p $\alpha\gamma\delta$ antibody, recognising all $\gamma\delta$ T cells irrespective of variable chain, and therefore is likely to have a different mode of action.

15

20

The assay was performed using skin-derived $\gamma\delta$ T cells obtained from three different donor samples (samples with 94%, 80% and 57% purity). Results are shown in **Figure 4**. EC50 values are summarised in **Table 4**, below.

5 **EXAMPLE 7: T cell degranulation assay**

A second assay assessed the degranulation of $\gamma\delta$ T cells. It is thought $\gamma\delta$ T cells may mediate target cell killing by perforin-granzyme-mediated activation of apoptosis. Lytic granules within the cytoplasm of the $\gamma\delta$ T cell may be released toward the target cell upon T cell activation. Therefore, labelling target cells with antibodies to CD107a and measuring the expression by
10 flow cytometry can be used to identify degranulating $\gamma\delta$ T cells.

As for **Example 6**, selected antibodies were tested against commercial anti-CD3 and anti-V δ 1 antibodies as positive controls or against 1252_P01_C08 as a positive control (for 1139_P01_E04, 1245_P02_F07, 1245_P01_G06 and 1245_P01_G09). IgG2a, IgG1 and
15 D1.3 antibodies were used as negative controls. The assay was performed using skin-derived $\gamma\delta$ T cells obtained from three different donor samples (samples with 94%, 80% and 57% purity). Results are shown in **Figure 5**. EC50 values are summarised in **Table 5**, below.

EXAMPLE 8: Killing assay

20 A third assay assessed the ability of $\gamma\delta$ T cells activated with the selected antibodies to kill target cells.

As for **Example 6**, selected antibodies were tested against commercial anti-CD3 and anti-V δ 1 antibodies as positive controls or against 1252_P01_C08 as a positive control (for 1139_P01_E04, 1245_P02_F07, 1245_P01_G06 and 1245_P01_G09) and anti-p $\gamma\delta$ as a
25 negative control. IgG2a, IgG1 and D1.3 antibodies were also used as isotype controls. The assay was performed using skin-derived $\gamma\delta$ T cells obtained from two donors (94% and 80% purity) and the results are shown in **Figure 6**.

30 Results from the three functional assays tested in **Examples 6-8** are summarised in **Table 5**.

Table 5. Summary of results obtained from functional assays

Clone ID	TCR downregulation (EC50 $\mu\text{g/ml}$ – 3 donors)	T cell degranulation (EC50 $\mu\text{g/ml}$ – 3 donors)	Killing assay (EC50 $\mu\text{g/ml}$ – 2 or 3 donors)
1245_P01_E07	0.04-0.11	0.007-0.004	0.06

1252_P01_C08	0.02-0.03	0.001-0.0006	0.02
1245_P02_G04	0.01-0.05	0.002	0.10
1245_P01_B07	Positive; 0.35 (1 donor only)	Positive; 0.1 (1 donor only)	0.13
1251_P02_C05	Positive; N/D	Positive; N/D	N/D*
1139_P01_E04	0.027-0.057	0.005	0.005-0.019
1245_P02_F07	0.032-0.043	0.001-0.002	0.006-0.018
1245_P01_G06	0.042-0.055	0.001	0.007-0.051
1245_P01_G09	0.029-0.040	0.001	0.003-0.008
1138_P01_B09	0.078-0.130	N/D	0.055-0.199
1251_P02_G10	0.849; N/D	N/D	N/D**
OKT3 (anti-CD3 antibody)	0.03-0.04	0.001-0.008	0.05
TS8.2 (anti-V δ 1 antibody)	0.48-0.8	0.07-0.16	N/D*

N/D: could not be determined; N/D*: could not be determined, titration curve did not reach plateau; N/D**: Reduced killing profile, EC50 not established

EXAMPLE 9: Epitope mapping

- 5 In order to determine the epitope of antigen/antibody complexes with high resolution, the protein complexes were incubated with deuterated cross-linkers and subjected to multi-enzymatic cleavage. After enrichment of the cross-linked peptides, the samples were analysed by high resolution mass spectrometry (nLC-LTQ-Orbitrap MS) and the data generated were analysed using XQuest (version 2.0) and Stavrox (version 3.6) software.

10

After trypsin, chymotrypsin, Asp-N, elastase and thermolysin proteolysis of the protein complex L1(DV1-GV4)/1245_P01_E07 with deuterated d0d12, the nLC-orbitrap MS/MS analysis detected 13 cross-linked peptides between L1(DV1-GV4) and the antibody 1245_P01_E07. Results are presented in **Figure 7**.

15

After trypsin, chymotrypsin, Asp-N, elastase and thermolysin proteolysis of the protein complex L1(DV1-GV4)/1252_P01_C08 with deuterated d0d12, the nLC-orbitrap MS/MS analysis detected 5 cross-linked peptides between L1(DV1-GV4) and the antibody 1252_P01_C08. Results are presented in **Figure 8**.

20

After trypsin, chymotrypsin, Asp-N, elastase and thermolysin proteolysis of the protein complex L1(DV1-GV4)/1245_P02_G04 with deuterated d0d12, the nLC-orbitrap MS/MS analysis detected 20 cross-linked peptides between L1(DV1-GV4) and the antibody 1245_P02_G04. Results are presented in **Figure 9**.

5

After trypsin, chymotrypsin, Asp-N, elastase and thermolysin proteolysis of the protein complex L1(DV1-GV4)/1251_P02_C05 with deuterated d0d12, the nLC-orbitrap MS/MS analysis detected 5 cross-linked peptides between L1(DV1-GV4) and the antibody 1251_P02_C05. Results are presented in **Figure 10**.

10

Epitope binding with another antibody, Clone ID 1141_P01_E01, was also tested. After trypsin, chymotrypsin, Asp-N, elastase and thermolysin proteolysis of the protein complex L1(DV1-GV4)/1141_P01_E01 with deuterated d0d12, the nLC-orbitrap MS/MS analysis detected 20 cross-linked peptides between L1(DV1-GV4) and the antibody 1141_P01_E01.

15 Results are presented in **Figure 11**.

A summary of the epitope mapping results is presented in **Table 6**.

Table 6. Results of epitope mapping for antigen/antibody complexes

Clone ID	Epitope mapping, amino acid numbering of SEQ ID NO: 1
1245_P01_E07	5, 9, 16, 20, 62, 64, 72, 77
1252_P01_C08	50, 53, 59, 62, 64
1245_P02_G04	37, 42, 50, 53, 59, 64, 68, 69, 72, 73, 77
1251_P02_C05	59, 60, 68, 72
1141_P01_E01	3, 5, 9, 10, 12, 16, 17, 62, 64, 68, 69

20

EXAMPLE 10: Expansion of V δ 1 T cells

Expansion of isolated $\gamma\delta$ T cells was investigated in the presence of selected antibodies and comparator antibodies. Comparator antibodies were selected from: OKT3 anti-CD3 antibody as a positive control, no antibody as a negative control or IgG1 antibody as an isotype control.

25 Commercially available anti-V δ 1 antibodies, TS-1 and TS8.2 were also tested for comparison.

Experiment 1:

An initial investigation was conducted by seeding 70,000 cells/well with Complete Optimizer and cytokines as described in the " $\gamma\delta$ T cell preparation" for blood-derived $\gamma\delta$ T cells of

30 Example 1. Selected and comparator antibodies were tested at various concentrations ranging

from 4.2 ng/ml to 420 ng/ml. This experiment was conducted using tissue culture plates which allow the binding/immobilisation of the antibodies to the plastic.

- Cells were harvested on days 7, 14 and 18 and the total cell count was determined using a cell counter (NC250, ChemoMetec). The results are shown in **Figure 12**. Cell viability of V δ 1 T cells was also measured on each harvest and all antibodies were shown to maintain cell viability throughout the experiments (data not shown). On day 18, the percentage, cell count and fold change of V δ 1 T cells was also analysed. The results are shown in **Figure 13**.
- As can be seen in **Figure 12**, the total number of cells produced in cultures with antibodies increased steadily throughout the culture and were comparable or better than the commercial anti-V δ 1 antibodies. At day 18, the proportion of V δ 1 positive cells in the presence of 1245_P02_G04 ("G04"), 1245_P01_E07 ("E07"), 1245_P01_B07 ("B07") and 1252_P01_C08 ("C08") antibodies at most concentrations tested was greater than in cultures where OKT3, TS-1 or TS8.2 control antibodies were present (see **Figure 13A**).

Experiment 2:

- A subsequent experiment was performed on isolated cells in a culture vessel with cytokines as described in the "V δ T cell preparation" of Example 1. Compared to Experiment 1, a different culture vessel was used whose surface does not facilitate antibody binding/immobilisation. Selected and comparator antibodies were tested at various concentrations ranging from 42 pg/ml to 42 ng/ml. During Experiment 2, results were obtained from experiments run in triplicates.
- Cells were harvested on days 7, 11, 14 and 17 and the total cell count was determined using a cell counter as before. The results are shown in **Figure 14**. On day 17, the percentage, cell count and fold change of V δ 1 T cells was also analysed. The results are shown in **Figure 15**.
- The cell composition, including non-V δ 1 cells, were also measured during Experiment 2. Day 17 cells were harvested and analysed by flow cytometry for surface expression of V δ 1, V δ 2 and $\alpha\beta$ TCR. The proportions of each cell type in each culture are shown graphically in **Figure 16** and the percentage values are provided in **Table 6**.

Table 7. Cell composition at day 17 - Percentage of live cells of each subset

	$\alpha\beta$ - V δ -	V δ 1	V δ 2	non V δ 1/V δ 2	$\alpha\beta$
no AB	63.00	18.17	0.86	7.10	0.37

OKT-3	25.63	50.43	0.25	20.13	1.13
IgG1	65.77	15.59	1.11	6.91	0.42
TS8.2 42ng/ml	30.60	53.57	3.59	7.46	0.14
TS-1 42ng/ml	18.77	65.90	0.91	9.51	0.12
C08 42ng/ml	0.79	96.43	0.08	2.51	0.05
C08 4.2ng/ml	1.91	94.67	0.18	2.63	0.05
C08 420pg/ml	8.47	80.57	0.28	8.42	0.04
C08 42pg/ml	35.97	25.93	3.04	19.50	0.31
B07 42ng/ml	0.94	95.57	0.46	2.73	0.05
B07 4.2ng/ml	1.79	94.10	0.40	3.28	0.01
B07 420pg/ml	3.08	91.80	0.29	3.94	0.02
B07 42pg/ml	17.93	62.90	0.85	9.16	0.07
E07 42ng/ml	2.29	85.13	0.19	11.65	0.04
E07 4.2ng/ml	2.15	91.23	0.13	5.77	0.04
E07 420pg/ml	9.25	73.90	0.42	13.05	0.02
E07 42pg/ml	49.23	18.67	2.17	7.70	0.43
G04 42ng/ml	1.90	88.53	0.47	8.09	0.05
G04 4.2ng/ml	4.25	89.67	0.93	3.98	0.02
G04 420pg/ml	25.97	50.60	1.45	12.72	0.11
G04 42pg/ml	44.00	13.77	2.33	26.30	0.32
C05 42ng/ml	25.00	42.03	3.75	13.67	1.32
C05 4.2ng/ml	46.87	22.03	2.58	16.46	0.38
C05 420pg/ml	33.53	44.60	2.23	11.13	0.22
C05 42pg/ml	36.83	25.23	6.16	18.00	0.30

- As can be seen from these results, the proportion of Vδ1 positive cells is greater in cultures with B07, C08, E07 and G04 present compared to OKT3, TS-1 or TS8.2 controls. Therefore, the tested antibodies produce and expand Vδ1 positive cells more efficiently than commercially available antibodies, even when present at low concentrations in culture.

Cells from day 17 of Experiment 2 were also analysed for additional cell markers, including CD3-CD56+ to identify the presence of Natural Killer (NK) cells and Vδ1 T cells which express CD27 (i.e. CD27+). The results are summarised in Table 7.

Table 8. Cell composition at day 17 - Percentage of NK and CD27+ cells

	% CD56+CD3-		%CD27+ of Vδ1	
	Mean	SEM	Mean	SEM
no AB	66.33	8.49	92.43	1.58
OKT-3	7.90	1.04	99.03	0.14
IgG1	70.67	6.41	87.87	0.81
TS8.2 42ng/ml	31.63	1.99	66.73	5.55
TS-1 42ng/ml	22.97	1.75	94.40	1.14
C08 42ng/ml	1.00	0.15	98.17	0.31
C08 4.2ng/ml	2.06	0.07	95.07	1.23
C08 420pg/ml	8.63	1.64	88.43	3.65
C08 42pg/ml	45.10	3.44	91.50	2.50
B07 42ng/ml	1.40	0.39	95.47	1.37
B07 4.2ng/ml	1.70	0.16	96.70	0.43
B07 420pg/ml	3.47	0.38	95.17	0.86
B07 42pg/ml	22.03	4.66	88.03	3.00
E07 42ng/ml	2.59	0.93	92.27	2.10
E07 4.2ng/ml	1.98	0.09	95.77	0.52
E07 420pg/ml	8.72	1.33	92.43	0.14
E07 42pg/ml	67.73	1.23	93.60	1.16
G04 42ng/ml	2.20	0.32	93.80	0.36
G04 4.2ng/ml	3.53	0.51	91.63	1.80
G04 420pg/ml	30.53	5.00	81.37	3.11
G04 42pg/ml	51.13	8.90	94.20	0.93
C05 42ng/ml	37.17	6.53	93.80	0.87
C05 4.2ng/ml	52.27	8.16	85.40	4.46
C05 420pg/ml	37.93	1.57	90.83	2.01
C05 42pg/ml	43.40	8.64	92.17	2.02

SEM: Standard error of the mean

EXAMPLE 11: Functionality of Vδ1 T cells

- 5 Vδ1 T cells expanded in the presence of the selected antibodies retained a polyclonal repertoire of CDR3 regions and were also tested for functionality using the SYTOX-flow killing assay. The results are presented for cells obtained during Experiment 1 at day 14 using cells

in a 10:1 Effector-to-Target (E:T) ratio (**Figure 17A**) and for cells obtained during Experiment 2 at day 17 (post freeze-thaw) using cells at a 1:1 and 10:1 E:T ratio (**Figure 17B**).

As can be seen in **Figure 17**, Vδ1 positive cells expanded in the presence of all antibodies effectively lysed target cells, indicating that they are functional even after freezing and thawing the cells.

EXAMPLE 12: Functionality of cells after storage

The functionality of cells after a storage step of freezing and then thawing was also investigated. A portion of cells was removed from culture at day 17 of Experiment 2 and frozen. Cells were then thawed and further expanded in culture with IL-15. **Figure 18** shows the total cell counts after 7 days of culturing cells post freeze-thaw for cultures contacted with B07, C08, E07, G04 or OKT-3 antibodies prior to freezing. All cultures showed the ability to proliferate after storage. Culturing was continued until day 42 and total cell counts were monitored during this period (results shown in **Figure 19**). Total cell numbers were maintained or increased in the cultures previously exposed to selected antibodies.

EXAMPLE 13: Binding equivalence studies on modified anti-Vδ1 antibodies

An ELISA-based antigen titration binding study was undertaken to compare the 1245_P02_G04 antibody manufactured in HEK to sequence and glycosylation variants thereof manufactured in CHO. Specifically, modifications to framework, to allotype, to hinge-mediated effector functionality, to Asn 297 glycosylation, and/or method of manufacture were undertaken and then included in this study. The assay ELISA set-up was as follows: Antigen comprised Antigen L1 (TRDV1/TRGV4); blocking buffer - 2% Marvel/PBS; mAbs diluted in a 1/2 dilution series starting at 5µg/ml; Antigen-antibody incubation in ELISA plates – 1 hour; Wash to remove non-specific binding - 3 x PBS-Tween, then 3 x PBS; Secondary antibody employed - DELFIA Eu labelled anti-human IgG (PerkinElmer; Cat #: 1244-330; 50 µg/ml) at 1/500 dilution; thereafter 1hr incubation prior to addition of DELFIA Enhancement Solution (PerkinElmer, used as per instruction); measurement by time-resolved fluorometry (TRF). For the antibodies made in CHO, standard expression vectors containing heavy and light chain cassettes were prepared under low-endotoxin conditions based on anion exchange chromatography. DNA concentration was determined by measuring the absorption at a wavelength of 260 nm. Sequences were verified with Sanger sequencing (with up to two sequencing reactions per plasmid depending on the size of the cDNA.) Suspension-adapted CHO K1 cells (originally from ATCC and adapted to serum-free growth in suspension culture) were employed for manufacture. The seed cells were grown in a chemically defined, animal-

component free, serum-free medium. Cells were then transfected with vectors and transfection reagent, and cells were grown further. Supernatant was harvested by centrifugation and subsequent filtration (0.2 µm filter) and the antibody was purified using MabSelect™ SuRe™ prior to formulation. To generate the example defucosylated antibody, protocols first described by von Horsten HH et al. (2010) Glycobiology 20(12):1607-18 were introduced into the CHO expression platform described above and ahead of expression and purification. Once manufactured and purified, mAb defucosylation was confirmed by MS-based analysis.

The results of this study are summarised in **Figure 20**. Therein the y-axis indicates ELISA signal and the x-axis indicates Vδ1 antigen concentrations (ug/ml) employed. Antibodies included in this titration study are outlined and for further detail, RSV = anti-RSV control mAb control (made in CHO). G04 = 1245_P02_G04 (made in HEK, SEQ ID NO:112). AD3 = variant G04 (made in CHO; SEQ ID NO:129 and comprising a variable domain sequence SEQ ID NO:131 and SEQ ID NO:132 and comprising a constant domain SEQ ID NO:133 and SEQ ID NO:134). AD4 = hinge modified AD3 (made in CHO; SEQ ID NO:130 and comprising a constant domain SEQ ID NO:133 and SEQ ID NO:135). AD3gly = defucosylated AD3 made in engineered CHO. Under all titrations, equivalent antigen binding was observed for all variants.

EXAMPLE 14: Anti-Vδ1 antibody binding equivalence studies on human germline Vδ1 antigen and a polymorphic variant thereof.

An ELISA-based binding study comparison was undertaken to study anti-Vδ1 antibody binding to human germline Vδ1 antigen as per IMGT database (see SEQ ID NO:1) versus binding to polymorphic human germline Vδ1 antigen (SEQ ID NO:128). Specifically, a comparison of antibody binding and cross-reactivity with Antigen L1 (containing canonical TRDV1/TRGV4 germline sequence) and L1AV (variant TRDV1/TRGV4 comprising said TRDV1 germline polymorphism) was performed. The results are presented in **Figure 21**. Antibodies indicated as follows: G04 = 1245_P02_G04 (SEQ ID NO:112); G04 LAGA = G04 with Hinge Fc modification (L235A, G237A EU numbering; SEQ ID NO: 136). E07 LAGA = 1245_P01_E07 with L235A, G237A, SEQ ID NO: 137. C08 LAGA = 1252_P01_C08 with L235A, G237A, SEQ ID NO:138, D1.3 = control. A serial dilution of each antibody to said antigens was performed and at all dilutions, for each antibody variant, equivalent binding to both antigens was observed. Shown is the equivalent binding observed for one example dilution (1nM antibody) in said series.

EXAMPLE 15: Anti-V δ 1 antibody binding conferred increase in V δ 1+ cell cytokine secretion

In brief, all antibodies were diluted to 10 μ g/ml and incubated overnight to bind the antibodies to the plate, before washing. Skin-derived $\gamma\delta$ T cells from two different skin donations were prepared as outlined elsewhere herein (see Example 1; specifically, the section on skin-derived $\gamma\delta$ T cell preparation). These skin cells were then added to tissue culture plates (100,000 cells per well) containing the bound antibodies as indicated. Cells were then left for one day prior to harvest of the supernatant and storage at -80°C. For cytokine analysis of the supernatants, an MSD U-PLEX Human Assay: K151TTK-1, K151UCK-1 was employed (Mesoscale Diagnostics, Maryland). Antibodies employed in this study included IgG1 (non-V δ 1-binding control), B07 (1245_P01_B07), E07 (1245_P01_E07), G04 (1245_P02_G04; 1245), and C08 (1252_P01_C08). The results of this study are presented in **Figure 22**. Specifically, **Figure 22 (A)** and **(B)** respectively outline the quantities of TNF-alpha and IFN-gamma detected in the supernatant when skin-derived $\gamma\delta$ T cells and the higher levels observed when anti-V δ 1 antibodies as indicated are applied

EXAMPLE 16: Anti-V δ 1 antibody conferred increase in V δ 1+ cell Granzyme B levels/activity

Skin-derived $\gamma\delta$ T cells were prepared as outlined elsewhere herein (see Example 1; the section on skin-derived $\gamma\delta$ T cell preparation). THP-1 cells were first loaded with GranToxiLux probe (a cell permeable, fluorogenic substrate is designed to detect Granzyme B activity in the target cells) and in accordance with manufacturer's instructions (Oncolmmunin, Inc. Gaithersburg, US). The THP-1 cells were then pulsed with the antibodies as indicated in **Figure 23** at 10 μ g/ml prior to mixing with the skin-derived $\gamma\delta$ T at a target/effector ratio of 1:20. The co-cultures were then briefly centrifuged to ensure rapid conjugate formation prior to co-culture for 1 hour and subsequent flow analysis in accordance with GranToxiLux protocols. Antibodies employed in this study included IgG1 (non-V δ 1-binding control), B07 (1245_P01_B07), E07 (1245_P01_E07), G04 (1245_P02_G04; 1245), and C08 (1252_P01_C08). The results are presented in **Figure 23** and highlight higher levels of Granzyme B in the target cancer cells observed when anti-V δ 1 antibodies as indicated are applied to this V δ 1+/THP-1 co-culture model system.

EXAMPLE 17: Anti-V δ 1 antibody conferred modulation and proliferation of immune cells in human tissue.

Human skin punch-biopsies (from five different donors) were incubated for 21-days in culture with the antibodies as indicated. Skin samples were prepared by removing subcutaneous fat etc. as described elsewhere herein (see Example 1; the section on skin-derived $\gamma\delta$ T cell preparation). Replicate punches from each donation were then placed on carbon matrix grids which were then placed in the well of a G-REX6 (Wilson Wolf). Each well was filled with complete medium as also described elsewhere herein. To investigate and compare the effect of differing antibodies, these were added on day 0, 7, 14 to a working concentration of 100ng/ml. After 21 days in culture cells were harvested and analysed by flow cytometry. The results of said study are presented in **Figure 24** and specifically highlight marked differences in modulatory effect of the differing antibodies: Order from left to right: Vd1 TS8.2 = TS8.2 (Thermo Fisher); OKT-3 (Biolegend); C08 IgG1 = 1252_P01_C08; E07 = 1245_P01_E07; G04 = 1245_P02_G04. **Figure 24 (A)** highlights the mean quantity of viable pan- $\gamma\delta$ TCR positive cells observed at end of culture; results presented as a gated fraction (percent mean + Std Dev) of the total live cell population as analysed by flow cytometry. For pan- $\gamma\delta$ content analysis flow gating strategy as follows: Singlets > Live cells> Pan- $\gamma\delta$ antibody (Miltenyi, 130-113-508). **Figure 24 (B)** highlights the mean quantity of viable V δ 1+ TCR positive cells observed at end of culture; results presented as a gated percent fraction (percent mean +Std Dev) of the total live cell population as analysed by flow cytometry. For V δ 1+ cell content analysis, flow gating strategy as follows: Singlets > Live cells> Pany δ (Miltenyi, 130-113-508) > V δ 1 (Miltenyi, 130-100-553). **Figure 24 (C)** highlights the number of viable double positive V δ 1+ CD25+ cells observed at end of culture; results presented as a gated fraction (percent mean +Std Dev) of the total live cell population. For CD25+ V δ 1+ cell content analysis, Flow gating strategy as follows: Singlets > Live cells> Pany δ (Miltenyi, 130-113-508) > V δ 1 (Miltenyi, 130-100-553) > CD25 (Miltenyi, 130-113-286. The combined results of this study summarise the differential effect of the antibodies of the invention as described herein relative to comparator antibodies TS8.2 and OKT3. And whilst not being bound by this theory, one possibility for the less favourable effects conferred on V δ 1+ cells by TS8.2 or OKT3 may be due to the deleterious effect conferred by these comparator molecules on immune cell function over time in this model system.

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EXAMPLE 18: Anti-V δ 1 antibody conferred modulation and proliferation of immune cells in TILs.

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Studies were undertaken to explore anti-V δ 1 antibody conferred modulation and proliferation of human tumour infiltrating lymphocytes (TILs). For these studies, human renal cell carcinoma (RCC) tumour biopsies were shipped fresh and processed upon receipt. Specifically, the tissue was chopped into $\sim 2\text{mm}^2$. Up to 1g of tissue was placed into each Miltenyi C tube along

with 4.7mL RPMI and enzymes from Miltenyi's Tumour Dissociation Kit at concentrations recommended by the manufacturer aside from Enzyme R which was used at 0.2 x concentration to prevent cleavage of pertinent cell surface molecules. C-Tubes were placed on the gentleMACS™ Octo Dissociator with Heaters. Program 37C_h_TDK_1 for the dissociation of soft tumours was selected. The digest was then filtered through a 70mM filter to generate a single cell suspension. RPMI containing 10% FBS was added to the digest to quench enzymatic activity. The cells are washed 2 x with RPMI/10%FBS and resuspended for counting. Derived cells were then seeded in TC wells (24-well G-REX, Wilson Wolf) at 2.5x10⁶ per well. Cells were then incubated without or without cytokines and with or without antibodies for 18 days. Antibodies included in the study are outlined in **Figure 25**. These include OKT3 (to 50ng/ml) and 1252_P01_C08 aka "C08" herein (to 500ng/ml). When included, bolus additions of these antibodies were added on day 0, 7, 11 and 14. During said incubation, media was replaced with fresh media on days 11 and day 14. Flow cytometry analysis was performed on day 0 and day 18 to determine the lymphocyte phenotype as well as fold change in cell number. Cells were first gated on live CD45+ cells and then as indicated. In arms where recombinant cytokines were included these were added as follows. Day 0: IL-4, IFN- γ , IL-21, IL-1 β . Additional IL-15 was included on day 7, 11, 14. Additional IL-21 and IFN- γ were included on day 7 and day 14 respectively. **Figure 25 (A)** shows the fold-increase in TIL V δ 1+ cells following 18 days culture in the presence of C08 or OKT3 with and without cytokine support (CK) where indicated.. These results show substantial fold increases in TIL V δ 1+ cells with the application of either the C08 or comparator OKT3 antibody in the presence of cytokines, as compared to antibody or cytokines alone. **Figure 25 (B)** shows increases in total V δ 1 cell number at harvest following . These results show substantial increases in TIL V δ 1+ cell number following culture with C08 or comparator OKT3 antibody in the presence of cytokines, as compared to antibody or cytokines alone. **Figure 25 (C)** presents an example gating strategy used in the flow cytometric analysis of the cells. From the live CD45+ cell population cells were gated on lymphocytes based on their forward and side scatter properties (not shown), $\gamma\delta$ T cells were then separated from $\alpha\beta$ T cells by staining for the T cell receptors. Finally, the proportion of V δ 1 cells within the total $\gamma\delta$ T cell population was determined. Example data for day 18 is shown for 2 conditions as indicated (+/-1252_P01_C08): 64.3% cells were CD45+, of those CD45% cells, 53.1% were $\gamma\delta$ +, and of the $\gamma\delta$ cells, 89.7% were V δ 1+. **Figure 25 (D)** presents a cell-surface phenotypic profile of TIL V δ 1+ cells at harvest. Higher levels of CD69 were observed following culture with the C08 antibody. **Figure 25 (E)** presents analysis of the TIL $\gamma\delta$ -negative, CD8-positive lymphocyte fraction within the live CD45-positive gate at harvest. In summary, the combined results highlight the modulatory effects conferred by anti-V δ 1 antibody of the invention described herein on TIL populations.

EXAMPLE 19: Anti-V δ 1 antibody conferred enhancement of V δ 1+ cell mediated cytotoxicity and diseased-cell-specific cytotoxicity.

Cytotoxicity/potency-assays and studies were undertaken in model systems comprising a triculture of V δ 1+ effector cells, THP-1 monocytic cancer cells, and healthy primary monocytes +/- anti-V δ 1 antibodies (1245_P02_G04; 1245_P01_E07; 1252_P01_C08) as described herein and inclusive of controls (no mAb or D1.3) as indicated in **Figure 26**. In brief, all antibodies were diluted to 10 μ g/ml in PBS and incubated overnight at 4 $^{\circ}$ C in 384-well ultra imaging assay plates (Perkin Elmer) to bind antibodies to the plate, before washing with PBS.

Healthy control monocytes were isolated from peripheral blood mononuclear cells (PBMCs; Lonza) by negative selection using magnetic activated cell sorting (MACS; Miltenyi Biotec). Monocytes and cultured THP-1 cells (ATCC) were stained with [0.5 μ M] CellTrace Violet and CellTrace CFSE live cell dyes respectively for 20 minutes before mixing in a 1:1 ratio. Expanded skin derived V δ 1 $\gamma\delta$ T-cells were detached from tissue culture flasks and serially diluted to generate a range of effector to target ratios (E:T) before adding to THP1:monocyte cell suspensions. Cell suspensions were seeded into 384-well assay plates to give a final cell seeding density of 1,000 THP-1 cells per well, 1,000 Monocytes per well, and a range of $\gamma\delta$ T-cells (top E:T ratio of 60:1). To determine the numbers of live THP-1 and healthy control monocytes after 24 hours, confocal images were acquired using an Opera Phenix high content platform capturing nine fields of view at 10x magnification. Live cell counts were quantified based on size, morphology, texture and intensity of live cell stains. Results are presented in **Figure 26**. **Figure 26 (A)** presents THP-1 and monocyte cell numbers after 24 hours in triple co-culture with $\gamma\delta$ T-cells in the presence of plate-bound mAbs or controls as indicated. Cell numbers were calculated using high content confocal microscopy using live cell imaging.

Figure 26 (B) presents a bar chart representation designed to highlight the window between diseased-cell specific killing and non-diseased healthy cell sparing at the top E:T ratio (60:1) after 24 hours co-culture: Left-hand bar chart; fold-increase in killing of diseased-cells (THP-1) versus killing of non-diseased cells (primary human monocytes). Right-hand bar chart; same data but represented as percent enhanced killing versus control. **Figure 26 (C)** presents tabulated results summarizing the percent improvement in potency of V δ 1 $\gamma\delta$ T-cells killing THP-1 target cells in the presence of V δ 1 mAbs compared to no mAb control as calculated from figure (A). **Figure 26 (D)** presents tabulated results of EC50 values as calculated from Figure (A) represented as $\gamma\delta$ T-cell numbers required to confer 50% THP-1 cell killing. The combined results and discoveries as outlined in **Figure 26** highlight the ability of antibodies described herein to enhance cytotoxicity and diseased-cell specificity of V δ 1+ cells.

EXAMPLE 20: Multi-specific antibody conferred enhancement of Vδ1+ effector cell mediated cytotoxicity; targeting a tissue-centric disease associated antigen.

Cytotoxicity/potency-assay studies were undertaken to explore the effect of multi-specific antibodies on co-cultures of Vδ1+ effector cells and A-431 cancer cells. A-431 (EGFR⁺⁺; ATCC) target cells were seeded in a 384-well imaging plate (Perkin Elmer) at 1,000 cells/ well and incubated at 37°C overnight in DMEM (10% FCS). Antibodies and multi-specific antibodies as indicated were diluted to 10µg/ml and added assay plate (2µg/ml final assay concentration). Expanded skin-derived Vδ1 γδ T-cells were detached from tissue culture flasks and serially diluted to give a range of E:T ratios (top E:T ratio of 60:1) before adding to assay plate. A-431 cells were incubated with Vδ1 γδ T-cells in the presence of antibodies or controls at 30°C, 5% CO₂. After 24 hours incubation, Hoechst 33342 (ThermoFisher) was added to stain cells (2µM final). To determine the numbers of live A-431 cells, confocal images were acquired using an Opera Phenix high content platform capturing nine fields of view at 10x magnification. Live cell counts were quantified based on size, morphology, texture, and intensity of live cell stains. Effector/Target (E:T) time course studies to determine the ET ratio wherein 50% of target cells are killed in model systems +/- the controls, comparators, antibodies and multi-specific antibodies as indicated. Results are presented in **Figure 27**.

First, **Figure 27 (A-D)** present example co-culture results wherein Vδ1+ / A-431 co-cultures were studied +/- multi-specific antibodies comprising anti-Vδ1 x anti-TAA (EGFr) bispecific binding moieties wherein the anti-Vδ1 VL+VH binding domain (to the first target) is combined with the CH1-CH2-CH3 domain of an anti-EGFr binding moiety (to the second target). Controls and comparators employed as indicated; from left to right: No mAb = no antibody added; D1.3 = D1.3 control; D1.3 IgG LAGA = D1.3 + L235A,G237A; D1.3 FS1-67 = D1.3 variable domain with EGFr binding constant domain plus L235A, G237A (SEQ ID NO: 139); Cetuximab (in-house generated). More specifically, **Figure 27 (A)** presents the results for five-hour co-cultures with aforementioned controls, comparators, and the following test articles: C08-LAGA = 1252_P01_C08 with L235A,G237A (SEQ ID NO:138); C08 FS1-67 = 1252_P01_C08 combined with EGFr binding domain containing a L235A,G237A (SEQ ID NO:140). **Figure 27 (B)** presents equivalent data of five-hour co-cultures with aforementioned controls, comparators, and the following test articles: G04-LAGA = 1245_P02_G04 with L235A,G237A; G04 FS1-67 = 1245_P02_G04 combined with EGFr binding domain containing L235A,G237A (SEQ ID NO:141). **Figure 27 (C)** presents equivalent data of five-hour co-cultures with controls, comparators, and the following test articles: E07-LAGA = 1245_P01_E07 with L235A,G237A; E07 FS1-67 = 1245_P01_E07 combined with EGFr binding domain containing

L235A,G237A (SEQ ID NO:142). **Figure 27 (D)** presents a Table summarizing the percent improvement in cytotoxicity of V δ 1 $\gamma\delta$ T-cells in the presence of controls, comparators, and test articles over 5, 12 and 24 hours. A greater than 450% enhancement can be observed when antibodies or fragment thereof as described herein are presented in a multi-specific format.

Second, **Figure 27 (E-H)** present example results wherein V δ 1+ / A-431 co-cultures were studied +/- multi-specific antibodies comprising anti-V δ 1 x anti-TAA (EGFr) bispecific binding moieties wherein the anti-V δ 1 binding domain (to the first target) comprises a full-length antibody (VH-CH1-CH2-CH3/VL-CL) then combined with an anti-EGFr scFv binding moiety (to the second target). Controls and comparators employed as indicated; from left to right: No mAb = no antibody added; D1.3 = Control; D1.3 IgG LAGA = D1.3 + L235A,G237A; D1.3 LAGA Cetuximab = D1.3 with L235A, G237A plus a C-term Cetuximab-derived scFv (SEQ ID NO:143); Cetuximab (in-house generated). More specifically, **Figure 27 (E)** presents five-hour co-cultures with aforementioned controls, comparators, and the following test articles: C08-LAGA = 1252_P01_C08 with L235A,G237A; C08 LAGA Cetuximab = 1252_P01_C08 with L235A,G237A and with C-term Cetuximab-derived scFv (SEQ ID NO: 144). **Figure 27 (F)** presents five-hour culture with aforementioned controls, comparators, and the following test articles: G04-LAGA = 1245_P02_G04 with L235A,G237A; G04 LAGA Cetuximab = 1245_P02_G04 with L235A,G237A and with C-term Cetuximab-derived scFv (SEQ ID NO: 145). **Figure 27 (G)** presents five-hour culture with controls, comparators, and the following test articles: E07-LAGA = 1245_P01_E07 with L235A,G237A; E07 LAGA Cetuximab = 1245_P01_E07 with L235A,G237A and with C-term Cetuximab-derived scFv (SEQ ID NO: 146). **Figure 27 (H)** presents a Table summarizing the percent improvement in potency of V δ 1 $\gamma\delta$ T-cells in the presence of controls, comparators, and test articles over 5, 12 and 24 hours. A greater than 300% enhancement can be observed when antibodies or fragment thereof as described herein are presented in a multi-specific format.

Third, **Figure 27 (I and J)** outline an example alternative approach to representing the data. Specifically shown is percentage improvement conferred by multi-specific antibody E07 FS1-67 (**I**) or C08 FS1-67 (**J**) upon V δ 1+ effector cell cytotoxicity towards EGFR+ cells relative at the 24-hour time point relative to all component parts and comparators as indicated.

EXAMPLE 21: Multi-specific antibody conferred enhancement of V δ 1+ mediated cytotoxicity and diseased-cell-specific cytotoxicity; targeting a hemopoietic-centric disease associated antigen.

Cytotoxicity/potency-assays and studies were undertaken in model systems comprising a triculture V δ 1+ effector cells, and Raji cancer cells, and healthy primary monocytes +/- multi-specific antibodies comprising anti-V δ 1 x anti-TAA (CD19) multi-specific antibody in order to determine whether tumour associated antigen (TAA) linked V δ 1 monoclonal antibodies in a bispecific format can enhance V δ 1 $\gamma\delta$ T-cell killing of specific target cells. Specifically, Raji cells (CD19++; ATCC) were incubated with V δ 1 $\gamma\delta$ T-cells in the presence of V δ 1 x CD19 multi-specific antibodies. All antibodies were diluted to 4 μ g/ml (final assay concentration 1 μ g/ml) and added to a 384-well imaging plate (Perkin Elmer). Expanded skin derived V δ 1 $\gamma\delta$ T-cells were detached from tissue culture flasks and serially diluted to give a range of effector to target ratios (E:T). Raji cells were stained with [0.5 μ M] CellTrace Far Red before mixing in a 1:1 ratio with titrated V δ 1 $\gamma\delta$ T-cells. Cell suspensions were seeded into 384-well assay plates to give a final cell seeding density of 1,000 Raji cells per well, and a range of $\gamma\delta$ T-cells (top E:T ratio of 30:1). To determine the numbers of live Raji after 24 hours, confocal images were acquired using an Opera Phenix high content platform capturing nine fields of view at 10x magnification. Live cell counts were quantified based on size, morphology, texture and intensity of live cell stains. Results are captured in **Figure 28**. Antibodies and comparators employed therein are as indicated. Specifically, RSV IgG = Motavizumab non-binding control, G04 = 1245_P02_G04; E07 = 1245_P01_E07; D1.3 VHVL = D1.3 HEL with heavy-chain C-terminal anti-CD19 scFv (see SEQ ID NO: 157 for scFv binding module employed); G04 VHVL = 1245_P02_G04 LAGA with heavy chain C-terminal anti-CD19 scFv (SEQ ID NO: 158); E07 VHVL = 1245_P01_E07 LAGA with heavy chain C-terminal anti-CD19 scFv (SEQ ID NO: 159). **Figure 28 (A)** Table summarizing (i) the calculated EC50s represented as $\gamma\delta$ T-cell numbers or E:T ratios required to induce 50% Raji cell killing, and (ii) percentage improvement in EC50s compared to no mAb control. **Figure 28 (B)** Bar chart representing the percentage improvement in ability of $\gamma\delta$ T-cells to lyse 50% of Raji target cells in the presence of V δ 1-CD19 multi-specific antibodies.