

Title: Immunoglobulins binding human V γ 9V δ 2 T cell receptors

Field of the invention: The present invention is in the field of medicine and relates to immunology. The invention relates to immunoglobulins binding T cells. In particular, the invention relates to immunoglobulins binding human V γ 9V δ 2 T cell receptors. The invention provides for immunoglobulin molecules that bind a human V γ 9V δ 2 T cell receptor, such as antibodies, single chain antibodies, or single domain antibodies, wherein the human V γ 9V δ 2 T cells may be modulated.

10 Background: The majority of $\gamma\delta$ peripheral blood lymphocytes (PBLs) in human adults express T-cell receptors (TCRs) comprising V γ 9 and V δ 2 regions. V γ 9V δ 2 T cells can react against a wide array of pathogens and tumour cells. This broad reactivity is understood to be conferred by phosphoantigens which are able to specifically activate this T-cell subset in a TCR dependent fashion. The broad antimicrobial and anti-tumour reactivity of V γ 9V δ 2 T-cells suggest a direct involvement in immune control of cancers and infections. In addition to fighting disease, in some diseases or medical treatment V γ 9V δ 2 T cells may be overstimulated or inadvertently activated.

Hence, agents that can activate V γ 9V δ 2 T cells can be useful in the treatment of infections or cancer as these may promote V γ 9V δ 2 T cell reactivity towards the pathogen or infected cells or cancer. Furthermore, agents that block activation of V γ 9V δ 2 T cells may be useful in diseases or medical treatment where it is advantageous to reduce V γ 9V δ 2 T cell activation, i.e. wherein V γ 9V δ 2 T cells are overstimulated or inadvertently activated. Finally, agents that can bind a V γ 9V δ 2 T cell, but do not have an effect on (phosphoantigen) activation of V γ 9V δ 2 T cells are useful for labelling cells, for example for selecting or identifying V γ 9V δ 2 T cells. Hence, there is a need in the art to provide for agents that can bind to V γ 9V δ 2 T cells, and for agents that can block phosphoantigen activation of V γ 9V δ 2 T cells or can activate V γ 9V δ 2 T cells.

Summary: The current disclosure now provides for novel agents that can bind to V γ 9V δ 2 T cells. The agents provided are immunoglobulins. The immunoglobulins provided bind to a V γ 9V δ 2 T cell receptor. Surprisingly, it was found that the immunoglobulins provided by the current disclosure have a substantial sequence identity. Hence, in one aspect, the present invention provides a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, comprising a CDR1 region, a CDR 2 region, and a CDR 3 region,

wherein the CDR1, CDR2 and CDR3 regions comprise:

- the amino acid sequences set forth in SEQ ID Nos: 4, 5 and 6, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 7, 8 and 9, respectively, or
- 5 - the amino acid sequences set forth in SEQ ID Nos: 4, 10 and 11, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 12, 13 and 14, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 15, 16 and 17, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 18, 19 and 20, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 18, 21 and 22, respectively, or
- 10 - the amino acid sequences set forth in SEQ ID Nos: 23, 24 and 25, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 15, 28 and 29, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 31, 32 and 33, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 31, 34 and 35, respectively, or
- the amino acid sequences set forth in SEQ ID Nos: 44, 45 and 46, respectively,
- 15 wherein the immunoglobulin molecule is an immunoglobulin molecule that activates human
 V γ 9V δ 2 T cells.

Also disclosed are human V γ 9V δ 2 T cell receptor binding immunoglobulin molecules,
comprising a CDR1 region and a CDR 2 region,

- 20 wherein the CDR1 region comprises an amino acid sequence with at least 40 % sequence
 identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; and wherein the

CDR2 region comprises an amino acid sequence with at least 60 % sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; wherein preferably the immunoglobulin molecule is a single domain antibody.

Furthermore, such immunoglobulins comprise a CDR3 region, wherein the CDR3
5 region contributes to V γ 9V δ 2 T cell receptor binding and may have an effect on the action of the immunoglobulin molecule. This may, without being bound by theory, implicate the CDR3 sequence in the functionality of the immunoglobulin molecule, i.e. type of modulation such as blocking activation of V γ 9V δ 2 T cells, inducing activation of V γ 9V δ 2 T cells or neither blocking activation nor inducing activation of V γ 9V δ 2 T cells. The immunoglobulins of the
10 disclosure are in particular for use in medical treatments and for use in assays involving V γ 9V δ 2 T cells.

Preferably, the immunoglobulin molecules according to the disclosure comprise a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 3, 6, 9, 11, 14, 17, 20, 22, 25, 27, 29,
15 30, 33, 35, 37, 40, 43, and 46. These CDR3 regions combined with the CDR1 and CDR2 sequences provided for binding and functions, as discussed in detail below.

In another aspect, there is provided a nucleotide sequence that encodes an immunoglobulin molecule as described herein.

In yet another aspect, there is provided a host cell comprising a nucleotide sequence
20 as described herein.

In yet another aspect, there is provided a method for preparing an immunoglobulin molecule as described herein, comprising:

- culturing a host cell as described herein;
- allowing the host cell to express the immunoglobulin;
- 25 - obtaining the immunoglobulin.

In yet another aspect, there is provided an immunoglobulin molecule as described herein for use in a medical treatment.

Also disclosed is a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that blocks activation of
30 human V γ 9V δ 2 T cells

Also disclosed is an immunoglobulin molecule as described herein for use in a medical treatment, wherein the medical treatment preferably comprises the use of inhibitors of the mevalonate pathway or wherein the medical treatment comprises the treatment of cancer or an infectious disease. Also disclosed is an immunoglobulin molecule as described
35 herein for blocking activation of human V γ 9V δ 2 T cells.

Also disclosed is immunoglobulin molecule as described herein, wherein the immunoglobulin molecule is an immunoglobulin molecule that activates human V γ 9V δ 2 T cells.

In yet another aspect, there is provided an immunoglobulin molecule as described herein
5 for use in a medical treatment, wherein preferably the medical treatment is a treatment of cancer.

In yet another aspect, there is provided non-therapeutic use of an immunoglobulin molecule as described herein for activating human V γ 9V δ 2 T cells.

In yet another aspect, there is provided an immunoglobulin molecule as described herein for activating human V γ 9V δ 2 T cells.

10 Also disclosed is an immunoglobulin molecule as described herein, wherein the immunoglobulin molecule is an immunoglobulin molecule that does not block activation of human V γ 9V δ 2 T cells; and does not activate human V γ 9V δ 2 T cells.

Also disclosed is the use of an immunoglobulin molecule as described herein for labelling
15 or for selecting human V γ 9V δ 2 T cells.

Figures:

Figure 1: Alignment of the VHH sequences wherein the framework regions (1, 2, 3 and 4) are indicated as well as CDR1, CDR2 and CDR3. The code for each of the VHHs is
20 indicated as well (i.e. 5C7 is the sequence of VHH 5C7).

Figure 2: VHH 5E7 and VHH 6F6 do not activate V γ 9V δ 2 T cells. Data indicate relative expression of the activation marker CD25, the pro-inflammatory cytokine IFN- γ , and the cytotoxic molecule granzyme B by healthy donor-derived V γ 9V δ 2 T cells in comparison with the positive control (phosphoantigen (pAg+) expressing HeLa cells)

25 Figure 3: VHH 5E7 neutralizes phosphoantigen induced activation of healthy donor-derived V γ 9V δ 2 T cells. A representative example demonstrates the dose dependent neutralization of phosphoAg-induced V γ 9V δ 2 T cell activation using VHH 5E7 while a non-specific VHH (negative control) cannot neutralize phosphoAg-induced V γ 9V δ 2 T cell activation. Vertical axis indicates activation of V γ 9V δ 2 T cells as assessed by CD25
30 expression, horizontal axis indicates different VHH concentrations. V γ 9V δ 2 T cell stimulations were performed using phosphoantigen expressing HeLa cells, generated by pretreating HeLa cells with increasing doses of the aminobisphosphonate pamidronate (which results in increasing levels of phosphoantigen expression by HeLa cells).

Figure 4: V γ 9V δ 2 TCR specific VHH are capable of inducing activation and cytokine
35 production in healthy donor-derived V γ 9V δ 2 T cells. Data indicate relative expression of the activation marker CD25 and the pro-inflammatory cytokine IFN- γ by healthy donor-derived V γ 9V δ 2 T cells in comparison with the positive control (phosphoantigen (pAg+) expressing

HeLa cells; standardized to 1) and a negative control VHH. Each bar represents an individual V γ 9V δ 2 TCR specific VHH; individual VHHs differ with respect to their capacity to induce activation and cytokine production in V γ 9V δ 2 T cells.

Figure 5: Dose dependent activation of healthy donor-derived V γ 9V δ 2 T cells. Data

5 indicate changes in CD25 expression (MFI) after 24 hr stimulation with increasing concentrations (10-100-500nM) of either a non-activating anti-V γ 9V δ 2 TCR VHH or an activating anti-V γ 9V δ 2 TCR VHH.

Figure 6: V γ 9V δ 2 TCR specific VHH can promote tumour cell death when fused to a tumor antigen specific VHH as a bispecific molecule. Representative example of experiment

10 in which V γ 9V δ 2 T cells were co-cultured overnight with the tumor cell line A431 in the presence (with) or absence (w/o) of a bispecific nanobody construct consisting of a tumor-antigen specific VHH fused to an activating anti-V γ 9V δ 2 TCR VHH. Data indicate CD25 expression (activation), and CD107a expression (degranulation) of V γ 9V δ 2 T cells and 7AAD+ tumor cells (indicating tumor cell death).

15 Figure 7: T cell receptor V γ 9 and/or V δ 2 binding specificity as determined using flow-cytometry: Representative flow-cytometric histogram indicates binding of a V γ 9V δ 2 TCR specific VHH (open histogram) and a negative control VHH (filled histogram) to V γ 9V δ 2 TCR expressing cells.

Figure 8: Clone VHH 5C7 does not activate healthy donor-derived V γ 9V δ 2 T cells nor

20 neutralize phosphoantigen induced activation of healthy donor-derived V γ 9V δ 2 T cells.

Representative example demonstrating no inhibitory nor activating effect of VHH 5C7 on

phosphoAg-induced V γ 9V δ 2 T cell activation. Vertical axis indicates activation of V γ 9V δ 2 T cells as assessed by CD25 expression, horizontal axis indicates different VHH

concentrations. V γ 9V δ 2 T cell stimulations were performed using phosphoantigen expressing

25 HeLa cells, generated by pretreating HeLa cells with increasing doses of the

aminobisphosphonate pamidronate (which results in increasing levels of phosphoantigen expression by HeLa cells).

Figure 9: Schematic of immunoglobulins.

A) A human antibody consisting of two heavy chains and two light chains; B) A single chain

30 antibody (or heavy chain only antibody) consisting of two single chains (or two heavy chains) that can dimerize via disulphide bridges, wherein each chain contains a variable domain.

Such a single chain antibody (or heavy chain only antibody) can be a llama antibody; C) A

single domain antibody contains one variable antibody domain e.g. of a single chain antibody (or heavy chain only antibody). A single domain antibody can consist only of the binding

35 region as depicted. The variable domain is indicated in grey, whereas the constant regions are indicated in white. The variable domain of the light chain is indicated in black.

Definitions:

In the following description and examples a number of terms are used. In order to provide a clear and consistent understanding of the specifications and claims and clauses, including the scope to be given to such terms, the following definitions are provided. Unless
5 otherwise defined herein, all technical and scientific terms used have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The disclosures of all publications, patent applications, patents and other references are incorporated herein in their entirety by reference.

Methods of carrying out the conventional techniques used in methods of the invention
10 will be evident to the skilled worker. The practice of conventional techniques in molecular biology, biochemistry, computational chemistry, cell culture, recombinant DNA, bioinformatics, genomics, sequencing and related fields are well-known to those of skill in the art and are discussed, for example, in the following literature references: Sambrook et al., Molecular Cloning. A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold
15 Spring Harbor, N. Y., 1989; Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1987 and periodic updates; and the series Methods in Enzymology, Academic Press, San Diego.

In this document and in its claims and clauses, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are
20 included, but items not specifically mentioned are not excluded. It encompasses the verbs "consisting essentially of" as well as "consisting of".

As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. For example, a method for isolating "a" DNA molecule, as used above, includes isolating a plurality of molecules (e.g. 10s, 100s, 1000s, 10s of
25 thousands, 100s of thousands, millions, or more molecules).

Aligning and alignment: With the term "aligning" and "alignment" is meant the comparison of two or more amino acid sequences based on the presence of short or long stretches of identical or similar amino acids. Several methods for alignment of amino acid sequences are known in the art, as will be further explained below. With the term "aligning"
30 and "alignment" is also meant the comparison of two or more nucleotide sequences based on the presence of short or long stretches of identical or similar nucleotides. Several methods for alignment of nucleotide sequences are known in the art, as will be further explained below.

"Expression of a gene" or "expression of a protein" refers to the process wherein a DNA region, which is operably linked to appropriate regulatory regions, particularly a
35 promoter, is transcribed into an RNA, which is capable of being translated by machinery of the cell into a protein or peptide (or active peptide fragment) that is encoded by the nucleotide sequence or which is active itself (e.g. in posttranscriptional gene silencing or RNAi).

As used herein, the term "operably linked" refers to a linkage of polynucleotide elements in a functional relationship. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter, or rather a transcription regulatory sequence, is operably linked to a coding sequence if it affects the transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary to join two or more protein encoding regions, contiguous and in reading frame.

The term "genetic construct" means a DNA sequence comprising a region (transcribed region), which is transcribed into an RNA molecule (e.g. an mRNA) in a cell, operably linked to suitable regulatory regions (e.g. a promoter). A genetic construct may thus comprise several operably linked sequences, such as a promoter, a 5' leader sequence comprising e.g. sequences involved in translation initiation, a (protein) encoding region, splice donor and acceptor sites, intronic and exonic sequences, and a 3' non-translated sequence (also known as 3' untranslated sequence or 3'UTR) comprising e.g. transcription termination sequence sites.

"sequence identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A. M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D. W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER; Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While a number of methods exist to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J. Applied Math (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in GUIDE TO HUGE COMPUTERS, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J. Applied Math (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., et al., Nucleic Acids Research (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S. F. et al., J. Molec. Biol. (1990) 215:403).

As an illustration, by an amino acid sequence with at least, for example, 70% "sequence identity" to a reference amino acid sequence of SEQ ID NO: 31 it is intended that the amino acid sequence is identical to the reference sequence except that the polypeptide

sequence may include up to 3 amino acid alterations per each of the 10 amino acids of the reference amino acid of SEQ ID NO: 31. Hence, the percentage of identity of an amino acid sequence to a reference amino acid sequence is to be calculated over the full length of the reference amino acid sequence. In other words, to obtain an amino acid sequence

5 comprising at least 70% identical to a reference amino acid sequence, up to 30% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 30% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference
10 amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

A "nucleic acid" or "nucleic acid sequence" according to the present invention may include any polymer or oligomer of pyrimidine and purine bases, preferably cytosine, thymine,
15 and uracil, and adenine and guanine, respectively (See Albert L. Lehninger, *Principles of Biochemistry*, at 793-800 (Worth Pub. 1982), which is herein incorporated by reference in its entirety for all purposes). The present invention contemplates any deoxyribonucleotide, ribonucleotide or peptide nucleic acid component, and any chemical variants thereof, such as methylated, hydroxymethylated or glycosylated forms of these bases, and the like. The
20 polymers or oligomers may be heterogeneous or homogenous in composition, and may be isolated from naturally occurring sources or may be artificially or synthetically produced. In addition, the nucleic acids may be DNA or RNA, or a mixture thereof, and may exist permanently or transitionally in single-stranded or double-stranded form, including homoduplex, heteroduplex, and hybrid states.

25 As used herein, the term "cancer" refers to or describes the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, breast cancer, colon cancer, lung cancer, prostate cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, thyroid cancer, renal cancer, carcinoma,
30 skin cancer, blood cancer, leukemia, melanoma, head and neck cancer, and brain cancer. As used herein, "cancer" is also referred to as malignant neoplasm.

The terms "amino acid sequence" or "protein" or "peptide" refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3 dimensional structure or origin. A "fragment" or "portion" of thereof may thus still be referred
35 to as an "amino acid sequence" or "protein" or "peptide". An "isolated amino acid sequence" is used to refer to an amino acid sequence which is no longer in its original natural environment, for example *in vitro* or in a recombinant bacterial or human host cell.

“T cells”, or “T lymphocytes”, belong to a group of white blood cells named lymphocytes, which play a role in cell-mediated immunity. T cells originate from hematopoietic stem cells in the bone marrow, mature in the thymus (that is where the T is derived from), and gain their full function in peripheral lymphoid tissues. During T-cell development, CD4⁻CD8⁻ T-cells (negative for both the CD4 and CD8 co-receptor) are committed either to an αβ or γδ fate as a result of an initial β or δ TCR gene rearrangement. Cells that undergo early β chain rearrangement express a pre-TCR structure composed of a complete β chain and a pre-TCRα chain on the cell surface. Such cells switch to a CD4⁺CD8⁺ state, rearrange the TCRα chain locus, and express a mature αβTCR on the surface. CD4⁻CD8⁻ T cells that successfully complete the γ gene rearrangement before the β gene rearrangement express a functional γδTCR and remain CD4⁻CD8⁻. (Claudio Tripodo et al. Gamma delta T cell lymphomas Nature Reviews Clinical Oncology 6, 707-717(December 2009). The T cell receptor associates with the CD3 protein complex. Mature T cells, i.e. expressing a αβTCR or a γδTCR, express the T cell receptor complex on the cell surface. The γδT-cells, which constitute about 1-5% of the total population of T cells in human peripheral blood, can be divided in further subpopulations. A subpopulation of γδT-cells constitutes Vγ9Vδ2 T-cells, which express a Vγ9Vδ2 TCR. Within the extracellular domain of a T cell receptor complementarity determining regions (CDR1, CDR2, CDR3) are located. These regions are in general the most variable domains and contribute significantly to the diversity among TCRs. CDR regions are composed during the development of a T-cell where so-called Variable-(V), Diversity-(D), and Joining-(J)-gene segments are randomly combined to generate diverse TCRs.

“Vγ9Vδ2 T-cells” are cells that may be functionally defined in that they are specifically and rapidly activated by a set of non-peptidic phosphorylated isoprenoid precursors, collectively named phosphoantigens. Phosphoantigens are produced by virtually all living cells. The most common phosphoantigen found in animal and human cells (including cancer cells) is isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). IPP is a metabolite from the mevalonate pathway. (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP or HMB-PP) is an intermediate of the non-mevalonate pathway of isoprenoid biosynthesis. HMBPP is an essential metabolite in most pathogenic bacteria, including *Mycobacterium tuberculosis*, as well as in parasitic protozoans, such as *Plasmodium* (causing malaria) and *Toxoplasma gondii*. Activation of Vγ9Vδ2 T-cells comprises clonal expansion, cytotoxic activity and expression of cytokines. “Vγ9Vδ2 T-cells” are also defined by expression of the Vγ9Vδ2 T-cell receptor. For example, cells may be selected using an antibody specific for the Vγ9Vδ2 T-cell receptor such as described below. These selected cells have undergone rearrangement of the γ and δ gene and encode a Vγ9 T-cell receptor chain and a Vδ2 T-cell receptor chain. From such selected cells, the nucleic acid (or amino acid) sequence corresponding to the Vγ9 T-cell receptor chain and the Vδ2 T-cell receptor chain may be determined.

The person skilled in the art is well capable of selecting and/or identifying cell populations characterized by expression of an antigen or receptor on the surface of the cell such as described throughout herein. It is understood that with regard to expression on the surface of cells, such as CD3, CD4, CD8, CD25, CD69, $\gamma\delta$ TCR and V γ 9V δ 2 TCR, this is typically done in a population of cells of which a portion of cells has a much higher level of expression of the antigen or receptor when compared to cells having a lower level of expression. Hence, the terms positive or negative are to be understood as being relative, i.e. positive cells have a much higher expression level as compared to cells being negative. Cells being negative in this sense may thus still have an expression level which may be detected.

Expression on the surface of cells may be analysed using Fluorescence Activated Cell Sorting (FACS), and many specific antibodies are commercially available, e.g. such as for CD3, CD4, CD8, CD25, CD69, $\gamma\delta$ TCR, V γ 9 TCR chain and V δ 2 TCR chain, that are suitable for such FACS analysis, such as described in the examples and as available. Such specific antibodies are immunoglobulins that bind with their respective antigen or receptor. V γ 9V δ 2 T-cells can hence also be defined and selected as being positive for V γ 9V δ 2 TCR in FACS. Antibodies suitable for FACS or similar separation techniques (such as e.g. antibodies conjugated to magnetic beads) are widely available. Conditions are selected, such as provided by the antibody manufacturer that allows the selection of negative and/or positive cells. Examples of antibodies that may be suitable for selection of V γ 9V δ 2 T cells, or engineered V γ 9V δ 2 T-cells such as available from BD Pharmingen (BD, 1 Becton Drive, Franklin Lakes, NJ USA) are V γ 9-PE (clone B3, #555733), V δ 2-FITC (clone B6, #555738), $\gamma\delta$ TCR-APC (clone B1, #555718) or such as available from Beckman Coulter is pan- $\gamma\delta$ TCR-PE (clone IMMU510, #IM1418U). Examples of antibodies that may be suitable for detecting CD25 and CD69 are CD25-PE (clone M-A251, #555432) and CD69-FITC (clone L78, #347823) available from BD Pharmingen.

Detailed description of the invention:

In a first aspect of the invention, a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule is provided, comprising a CDR1 region and a CDR 2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40 % sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; wherein the CDR2 region comprises an amino acid sequence with at least 60 % sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; wherein preferably the immunoglobulin molecule is a single domain antibody.

A human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule according to the invention, is an immunoglobulin molecule that binds e.g. to a V γ 9V δ 2 T cell receptor such as defined by the amino acid sequences of the V γ 9 and V δ 2 T cell receptor chains as listed in

SEQ ID NO. 71 and 72. Binding to such a T cell receptor can be detected e.g. via FACS analysis, such as described in the example section. For example, cells expressing a V γ 9V δ 2 T cell receptor, e.g. SEQ ID NO. 71 and 72, are contacted with either a control immunoglobulin molecule or an immunoglobulin molecule binding to a V γ 9V δ 2 T cell receptor. Alternatively, V γ 9V δ 2 T cells derived from a healthy human donor as described in the examples can be contacted with either a control immunoglobulin molecule or an immunoglobulin molecule binding to a V γ 9V δ 2 T cell receptor. The amount of immunoglobulin bound to the cell is increased when the specific immunoglobulin molecule is compared with a control immunoglobulin molecule that does not bind to a V γ 9V δ 2 T cell receptor (see for example figure 7). A human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule according to the invention can be defined e.g. as being an immunoglobulin that results in a minimal 2-fold increase in mean-fluorescence intensity (MFI), relative to a control immunoglobulin, as determined by flow cytometry. The MFI is the mean of the fluorescence intensity in the fluorescence channel that is chosen (FITC, PE, PerCP, etc.). As a negative control antibody a single domain antibody (or VHH, nanobody) against azo-dye reactive red 6 (RR6) can be used (Spinelli S et al, Biochemistry 2000;39:1217-1222). Hence, the skilled person is well capable of selecting appropriate conditions to determine binding of an immunoglobulin molecule with the V γ 9V δ 2 T cell receptor. Immunoglobulin binding can be expressed in terms of specificity and affinity. The specificity determines which antigen or epitope thereof is bound by the immunoglobulin molecule.

An "immunoglobulin molecule" (abbreviated as "Ig") as used herein is well-known in the art and comprises the term "antibody". The term "immunoglobulin" as used herein refers to any polypeptide comprising an antigen-binding site with complementarity determining regions (CDR). The term includes, but is not limited to antibodies, monoclonal antibodies, monospecific antibodies, multispecific antibodies, humanized antibodies, chimeric antibodies, human antibodies, single chain antibodies, heavy chain only antibodies, llama antibodies, single domain antibodies and nanobodies (e.g. VHH). The term "immunoglobulin molecule" may also include immunoglobulin fragments such Fab, F(ab')₂, Fv, scFv, Fd, dAb, and other antibody fragments or other constructs comprising CDRs that retain antigen-binding function. Typically, such fragments comprise an antigen-binding domain. The immunoglobulin molecules or fragments thereof may be any of the known antibody isotypes and their conformations, for example, IgA, such as IgA1 or IgA2, IgD, IgE, IgG, such as IgG1, IgG2a, IgG2b, IgG3, IgG4, or IgM class, or may constitute mixtures thereof in any combination, such as a mixture of antibodies from the IgG1 and IgG2a class.

Immunoglobulins are immune system-related proteins. Human antibodies consist of four polypeptides—two heavy chains and two light chains joined to form a "Y"-shaped molecule (see figure 9A). The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. Each of the tips has a specificity for binding antigen. The variable region

of human antibodies includes the ends of the light and heavy chains, i.e. the variable domains of the light and heavy chains. The constant region determines the mechanism used to e.g. activate the immune system.

Antibodies are divided into five major classes, IgM, IgG, IgA, IgD, and IgE, based on
5 their heavy chain constant region structure and immune function. Also subclasses of the heavy chain are known. For example, IgG heavy chains in humans can be any of the IgG1, IgG2, IgG3 and IgG4 subclasses.

Each chain, i.e. immunoglobulin molecule, has a variable domain. The variable domain of immunoglobulin molecules is subdivided into hypervariable (HV) and framework
10 (FR) regions. HV regions have a high ratio of different amino acids in a given position, relative to the most common amino acid in that position. The hypervariability regions are referred to as complementarity determining regions (CDR). Immunoglobulin molecules have three complementarity determining regions (CDR1, CDR2 and CDR3). Four framework regions, with much less variable amino acids sequences, separate the CDR regions. The CDR regions
15 can direct binding to the antigen, such as a V γ 9V δ 2 T cell receptor (see for example figure 1, wherein the framework regions and CDR regions are indicated of the selected VHHs). The framework regions form a beta-sheet structure which serves as a scaffold to position the CDR regions to contact the antigen.

Llama antibodies consist of two heavy chains (see figure 9B). Each of the heavy
20 chains is an immunoglobulin molecule with a single variable domain. Such an antibody is referred to as a single chain antibody, i.e. it comprises one type of chain. Such an antibody can also be referred to as a heavy chain only antibody.

A single domain antibody is an immunoglobulin molecule containing a single monomeric variable domain (see figure 9C). Single domain antibodies thus contain a single
25 CDR1, a single CDR2 and a single CDR3. A single domain antibody can be derived from a single chain antibody (or heavy chain only antibody). Like a whole antibody, a single domain antibody is able to bind selectively to a specific antigen. Single domain antibodies may contain only the variable domain of an immunoglobulin chain having CDR1, CDR2 and CDR3 and framework regions, such antibodies can also be referred to as VHHs or nanobodies. With
30 a molecular weight of only about 12–15 kDa, nanobodies are much smaller than common antibodies (150–160 kDa) which are composed of two heavy chains and two light chains.

CDR1, CDR2 and CDR3 sequences may be exchanged between species. For example, from a llama immunoglobulin molecule, CDR sequences may be selected and exchanged with CDR sequences in a human immunoglobulin molecule, to obtain a human
35 immunoglobulin molecule having the specificity that is derived from the llama CDR sequences. This may be advantageous as a human sequence may be less immunogenic to humans as compared to the original llama framework sequence. Such an exchange of CDR sequences is known as humanization. Hence, the immunoglobulin molecules, single chain

antibodies and single domain antibodies according to the invention may have human derived immunoglobulin sequences or llama derived immunoglobulin sequences and have the CDR1, CDR2 and CDR3 sequences replaced with the CDR sequences according to the invention in order to provide for human V γ 9V δ 2 T cell receptor binding. For example, a single chain

- 5 human antibody may comprise a sequence corresponding to the human heavy chain sequence but has been mutated, e.g. having the CH1 domain deleted, such that a llama-like single chain antibody is formed (see e.g. figure 9B), and has the CDR regions of said human heavy chain sequence replaced by the CDR sequences according to the invention. A human immunoglobulin, a human single chain antibody or a human single domain antibody hence
10 refers to the origin of framework and/or constant regions and not to the origin of the CDR1, CDR2 and CDR3 regions of the invention.

As described in the example section, human V γ 9V δ 2 T cell receptor binding immunoglobulin molecules were selected by using a strategy involving immunizing Llama
glamas with human donor-derived V γ 9V δ 2 T cells and phage display. The VHH sequences
15 that were selected were sequenced and are listed in table 3 and depicted in figure 1. The CDR1, CDR2 and CDR3 regions of the selected VHHs are listed below in table 1. Each of the CDRs is also listed in table 3 with the corresponding SEQ ID NO.

Table 1. Of the 20 VHHs that were selected the CDR1, CDR2 and CDR3 sequences are
20 listed. The Ref. CDR1 and CDR2 are listed above (corresponding to SEQ ID NO. 31 and SEQ ID NO.32 respectively), and the sequence identify (%) of each CDR1 and CDR2 region with the respective Ref. CDR1 and CDR2 is listed as well.

		CDR1	%	CDR2	%	CDR3
Nr	Ref	GRTFSNYAMG		AISWSGGSTYYADSVKG		
1	5C7	GRTFSRYTMG	80	AISWSGGRTNFAGSVKG	71	DWLVPVPGRESYDY
2	5E3	GRTFSSYAMG	90	AISWSGGTTYADSVKG	94	SLDCSGPGCHTAEYDY
3	6H1	GRTFSEYAMG	90	AISWTGSKTYADSVKG	82	SSDCSGPGCHTEEYDY
4	5G3	GRTFSSYAMG	90	AVSWSGGSTYYADSVKG	94	SQDCSGPGCYTNEYDS
5	5C1	GSIFSNYAMA	70	AVSWSGGRTYYADSVKG	88	SLSCSGPGCSLEEYDY
6	5D3	GRPFSNYAMG	90	VISWSGGSTYYADSVKG	94	QFSGASTVVAGTALDYDY
7	6E3	GRPFSNYGMG	90	GISWSGGSTDYADSVKG	88	VFSGAETAYYPSDDYDY
8	6H4	GRPFSNYGMG	90	GISWSGGSTDYADSVKG	88	VFSGAETAYYPSDDYDY
9	6C1	GRPFSNYGMG	90	GISWSGGSTDYADSVKG	94	VFSGAETAYYPSDDYDY
10	6H3	GRPFSNYGMG	90	GITWSGGSTHYADLVKG	76	VFSGAETAYYPSTEYDY
11	6G3	GRPFNNYGMG	70	GISWSGGSTYYADSVKG	94	VFSGAETAQYPSYDYDY
12	6F6	GRPFSNYAMG	90	AVTWSGGSTYYADSVKG	88	QFNGAENIVPATTTPTSYDY
13	5C8	GRPFSNYAMG	90	AISWSGGSTSYADSVKG	94	QFSGADYGFGRLGIRGYEYDY

14	5E7	GRPFSNYAMG	90	AISWSGGSTSYADSVKG	94	QFSGADYGFGRLGIQGYEYDY
15	5F5	GRTFSNYAMG	100	AISWSGGSTYYADSVKG	100	MFSGSESQLVVVITNLYEYDY
16	6A1	GRTFSNYAMG	100	TISWSGGSTYYADSVKG	94	AFSGSDYANTKKEVEYDY
17	5D7	GRTFSNYAMG	100	AISWSGGMTDHADSVKG	82	AFAGDIPYGSSWYGDPPTYDY
18	5B11	GRTSSTFSMA	50	AINWSGGSTRYADSVSD	76	RRGGIYYSTQNDYDY
19	6C4	VRTFSDYRMG	70	TISWSGGLTYADSVKG	94	GGGYAGGTYYPHEE
20	6E4	GFTFDDYCIA	40	CITTSDBGSTYYADSVKG	76	YFGYGCYGGAQDYRAMDY

The immunoglobulins that were selected by the inventors to bind the human V γ 9V δ 2 T cell receptor surprisingly had a substantial sequence identity with regard to CDR1 and CDR2. Without being bound by theory, such CDR1 and CDR2 sequences substantially contribute to the binding of the V γ 9V δ 2 T cell receptor. More variability was found for the CDR3 region, which, without being bound by theory, may implicate the CDR3 sequence in the functionality of the immunoglobulin molecule, i.e. type of modulation such as blocking activation of V γ 9V δ 2 T cells, inducing activation of V γ 9V δ 2 T cells or neither blocking activation nor inducing activation of V γ 9V δ 2 T cells. Hence, the immunoglobulin molecule comprises a CDR1 region and a CDR2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40% sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG, and wherein the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG. Preferably the CDR2 region comprises an amino acid sequence with at least 70% sequence identity with the amino acid sequence of SEQ ID NO. 32.

Preferably, the immunoglobulin molecule is a single chain antibody. As said, the immunoglobulins are derived from llama. Llamas produce antibodies with a single heavy chain that dimerizes via disulphide bridges, i.e. a llama antibody has two single variable domains from two chains (see figure 9B).

In one embodiment, the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with SEQ ID NO. 32 AISWSGGSTYYADSVKG, wherein the said amino acid sequence has a T at position 9, an A at position 12, and a V at position 15. When the sequences of the selected CDR2 regions are compared, the amino acids at these positions do not show variation. Hence, without being bound by theory, these positions appear to be of importance to binding the V γ 9V δ 2 T cell receptor. It is understood that the position referred to relates to the position in the reference sequence and does not refer to the position in the immunoglobulin molecule as a whole. Hence, the CDR2 region has identical amino acids to SEQ ID NO.32 at the specified position.

As described in the example section, the CDR1, CDR2 and CDR3 regions were selected from llama antibodies. Hence, a single chain antibody according to the invention may comprise immunoglobulin molecule sequences that are derived from the llama. It is

understood that in such a llama single chain antibody, the original CDR sequences are replaced by replacement CDR sequences, e.g. such as listed in table 1, to arrive at a llama single chain having the specificity of the replacement CDR sequences. Similarly, the same may be done with a human heavy chain sequences. The human single chain antibody than
5 having the specificity being governed by the replacement CDR sequences. Transferring CDR1, CDR2 and CDR3 regions to other frameworks, e.g. to other species such as human frameworks is well known in the art.

In one embodiment, the single chain antibody is a single domain antibody. Single chain antibodies comprise framework regions. Hence, a human single domain antibody may
10 have human framework regions, e.g. derived from either a human heavy and/or human light chain sequence and CDR1, CDR2 and CDR3 sequences according to the invention. A llama single domain antibody has llama framework regions.

In one embodiment, one or more of the framework regions are selected from the group of amino acid sequences of SEQ ID NO. 67-70. These framework regions are the
15 framework regions from one of the VHH clones that was isolated. As can be observed, the framework regions from the 20 isolated clones do not vary substantially.

In one embodiment, the immunoglobulin molecule, the single chain antibody or the single domain antibody comprises a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID
20 NO. 3, 6, 9, 11, 14, 17, 20, 22, 25, 27, 29, 30, 33, 35, 37, 40, 43, and 46. These CDR3 regions combined with the CDR1 and CDR2 sequences provided for binding and function, as discussed below.

In one embodiment, the immunoglobulin molecule, the single chain antibody or the single domain antibody has the combinations of the amino acid sequences of the CDR1,
25 CDR2 and CDR3 regions from the antibodies such as listed in table 1. In one embodiment, the immunoglobulin molecule, the single chain antibody or the single domain antibody comprises an amino acid sequence selected from the group of amino acid sequences consisting of SEQ ID NO. 47-66.

In one embodiment, an immunoglobulin molecule according to the invention as
30 disclosed above is provided for use in a medical treatment. It is understood that a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule when it binds a human V γ 9V δ 2 T cell in vivo, e.g. in a medical treatment, that it may not be desirable that the immunoglobulin molecule is a fully functional immunoglobulin molecule as upon binding to human V γ 9V δ 2 T cells it may trigger an immune response directed against the human V γ 9V δ 2 T cells. Hence,
35 in such a scenario, immunoglobulin molecules that do not have functional constant regions, i.e. inactivated or deleted, are preferred such as e.g. in nanobodies and VHHs. This may be in particular useful when the action of the human V γ 9V δ 2 T cells is required in vivo.

In one embodiment, a nucleotide sequence is provided that encodes an immunoglobulin molecule according to the invention. The sequences as disclosed herein relate to amino acid sequences. Hence, the skilled person is well capable of providing for a nucleotide sequence encoding an amino acid sequence, as it only requires to use a codon
5 table to convert amino acid sequence into nucleotide sequence. Such nucleotide sequence may be used to operably link it to promoter sequences, polyA signals etc., to provide for a genetic construct with which the antibody may be expressed. Such a genetic construct comprising the nucleotide sequence may be comprised in a host cell.

In one embodiment, a method is provided for preparing an immunoglobulin molecule
10 according to the invention comprising:

- culturing a host cell according to the invention comprising a nucleotide sequence that encodes an immunoglobulin molecule according to the invention;
- allowing the host cell to express the immunoglobulin;
- obtaining the immunoglobulin.

15 Furthermore, the invention also provides for a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that blocks activation of human V γ 9V δ 2 T cells. Blocking activation of human V γ 9V δ 2 T cells is advantageous in conditions and/or treatments wherein activation of human V γ 9V δ 2 T cells is undesirable.

20 V γ 9V δ 2 T cells can be strongly and specifically activated by small nonpeptidic phosphorylated intermediates, referred to as phosphoantigens (pAg) from the mammalian mevalonate pathway or the microbial deoxyxylulose-phosphate pathways. Phosphoantigens can then be specifically recognized (resulting in activation) by V γ 9V δ 2 T cell through interaction between pAg and membrane bound butyrophilin3A1/CD277 molecules. V γ 9V δ 2 T
25 cell receptor binding immunoglobulin molecules, as shown in the examples, can block phosphoantigen induced activation of V γ 9V δ 2 T cells.

Preferably, the human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that blocks activation of human V γ 9V δ 2 T cells, is a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule,
30 comprising a CDR1 region and a CDR 2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40% sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; and wherein the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; and wherein preferably the immunoglobulin molecule is a
35 single chain antibody. In one embodiment, the CDR2 region of said immunoglobulin molecule comprises an amino acid sequence with at least 60 % sequence identity with SEQ ID NO. 2 AISWSGGSTYYADSVKG, wherein the said amino acid sequence has a T at position 9, an A at position 12, and a V at position 15. In a further embodiment, the immunoglobulin molecule

is a single domain antibody, preferably wherein the single domain antibody is derived from a llama single chain antibody or a human single chain antibody. In a further embodiment, the immunoglobulin molecule is a single chain antibody or a single domain antibody. In further embodiments, the immunoglobulin molecule or the single chain antibody or the single domain antibody, comprises one or more of the framework regions selected from the group of amino acid sequences of SEQ ID NO. 67-70.

In one embodiment, the said human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule that blocks activation of human V γ 9V δ 2 T cells is for use in a medical treatment. In a further embodiment, said immunoglobulin molecule is for use in a medical treatment, wherein the medical treatment comprising the use of inhibitors of the mevalonate pathway or wherein the medical treatment comprises the treatment of cancer. In another further embodiment said immunoglobulin molecule is for use in a medical treatment wherein the medical treatment comprises the treatment of an infectious disease.

Inhibitors of the mevalonate pathway that act downstream of pAg production, that include commonly clinically prescribed aminobisphosphonates such as pamidronate, alendronate, risedronate, ibandronate and zoledronate. Another class of compounds includes alkylamines such as isobutylamine, isoamylamine, and n-butylamine. Such compounds can be used for the treatment of Paget's disease, osteoporosis, hypercalcemia, and prevention of skeletal events in case of malignant bone metastases. This results in the intracellular accumulation of the endogenous pAg isopentenyl-pyrophosphate (IPP) and the subsequent selective activation and expansion of V γ 9V δ 2 T cells. Aminobisphosphonate administration is frequently accompanied by an acute febrile response due to this selective activation of V γ 9V δ 2 T cells. This acute phase response has a peak onset of 1 day and a median duration of 3 days and mostly consists of fever, chills, flushes, acute musculoskeletal symptoms, pain, generalized discomfort and local complaints involving the back, neck, chest or shoulders, nausea, vomiting, and diarrhea. Hence, in a medical treatment, said human V γ 9V δ 2 T cell receptor binding immunoglobulin molecules that block activation of human V γ 9V δ 2 T cells can prevent the acute phase response induced by e.g. aminobisphosphonate administration in patients with Paget's disease, osteoporosis, bone metastases, and hypercalcemia. Furthermore, such immunoglobulin molecules may also be advantageous in the medical treatment of excessive activation of V γ 9V δ 2 T cells in vivo, which can occur for example during an infection where V γ 9V δ 2 T cells are overstimulated or chronically stimulated or in certain cancerous conditions where chronic overactivity of the mevalonate pathway in tumour cells can result in V γ 9V δ 2 T cell exhaustion. Such (over)stimulation can be measured in patients for example by measuring an increase in V γ 9V δ 2 T cells as compared to baseline levels, or by measuring supranormal levels of V γ 9V δ 2 T cells, e.g. more than 5% of the T cells are V γ 9V δ 2 T cells, combined with an upregulation of surface markers such as CD69 (early activation marker) or CD25 (late activation marker) on V γ 9V δ 2 T cells. It is understood

that due to migration of the V γ 9V δ 2 T cells out of the blood to tissues, measuring supranormal levels of V γ 9V δ 2 T cells is not a requirement. On the other hand, in chronic overstimulation, V γ 9V δ 2 T cells may be less well activated, and that can be a sign of overstimulation as well. Cytokine production (IFN-gamma, TNF-alpha) and cytotoxic granule content can also be measured intracellularly by flow cytometry.

In a preferred embodiment, the said human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule that blocks activation of human V γ 9V δ 2 T cells, is an immunoglobulin molecule comprising a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 27 and 30.

In one embodiment, the said human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule that blocks activation of human V γ 9V δ 2 T cells, is used for blocking activation of human V γ 9V δ 2 T cells. According to this embodiment, said immunoglobulin molecule (which includes the single chain antibody or single domain antibody), is used in assays, e.g. such as described in the examples, to block activation.

The invention also provides an immunoglobulin molecule that activates human V γ 9V δ 2 T cells, that is a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, comprising a CDR1 region and a CDR2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40% sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; and wherein the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; and wherein preferably the immunoglobulin molecule is a single chain antibody. In one embodiment, the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with SEQ ID NO. 2 AISWSGGSTYYADSVKG, wherein the said amino acid sequence has a T at position 9, an A at position 12, and a V at position 15. In a further embodiment, the immunoglobulin molecule is a single domain antibody, preferably wherein the single domain antibody is a llama single chain antibody or a human single chain antibody. In a further embodiment, the single chain antibody is a single domain antibody. In further embodiments, the immunoglobulin molecule or the single chain antibody or the single domain antibody, comprises one or more of the framework regions selected from the group of amino acid sequences of SEQ ID NO. 67-70.

Preferably, said immunoglobulin molecules that activate human V γ 9V δ 2 T cells comprises a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 6, 9, 11, 14, 17, 20, 22, 25, 29, 33, 35, and 46.

Current strategies that aim to exploit V γ 9V δ 2 T cells depend on their systemic activation (e.g. by aminobisphosphonates or synthetic phosphoantigens such as BrHPP) or on e.g. adoptive transfer of V γ 9V δ 2 T cells. These approaches have been shown to be well

tolerated by patients and signs of antitumor activity have been documented. However, results are not consistent enough to allow widespread clinical application. Described strategies result in systemic activation of V γ 9V δ 2 T cells, but do not result in the specific recruitment of these cells to the tumour, where they are supposed to exert their antitumor effect. The said

5 immunoglobulin molecule that activates human V γ 9V δ 2 T cells and that are preferably linked to an agent can be used to activate V γ 9V δ 2 T cells in a clinical setting.

Preferably, said immunoglobulin molecule that activates human V γ 9V δ 2 T cells are linked to an agent. Said agent is preferably an agent that can bind to a target, e.g. a cancer cell or an infected cell, e.g. infected with a virus, or a non-host cell, e.g. bacteria. Preferably
10 said agent is an immunoglobulin molecule. More preferably, said immunoglobulin molecule is a single chain antibody or a single domain antibody. By linking to an agent, the agent can recruit and activate the human V γ 9V δ 2 T cells at the site where the action of the human V γ 9V δ 2 T cells is required, in contrast to systemic activation. For example, said immunoglobulin molecule that activates human V γ 9V δ 2 T cells can be linked to a tumour-
15 specific antibody, an antiviral antibody, or an antibacterial antibody. Such a tumour specific antibody can be any antibody. Such an immunoglobulin molecule linked to another antibody can be referred to as a bispecific antibody. A bispecific antibody may also consist of a first immunoglobulin molecule comprising the CDR1, CDR2 and CDR3 regions according to the invention, which is a chain such as comprised in a single chain antibody, wherein the first
20 immunoglobulin chain is paired with a second immunoglobulin molecule which is also a chain such as being comprised in a single chain antibody wherein the second immunoglobulin binds to the target. A bispecific antibody is thus formed that has two chains (similar to as depicted in figure 9B), each chain having a different single binding domain wherein one binding domain comprises CDR1, CDR2 and CDR3 in accordance with the invention and the other binding
25 domain binds to the targets. Said immunoglobulin molecule that activates human V γ 9V δ 2 T cells and linked to an agent can also be a bispecific antibody that comprises two single domain antibodies, the first single domain antibody comprising the CDR1, CDR2 and CDR3 regions according to the invention, wherein the first single domain antibody is linked to a second single domain antibody wherein the second single domain antibody binds to the
30 target.

Hence, in one embodiment, said immunoglobulin molecule that activates human V γ 9V δ 2 T cells, and which is preferably linked to an agent is for use in a medical treatment. Preferably the medical treatment is a treatment of cancer or of an infection.

In a further embodiment, the use is provided said immunoglobulin molecule that
35 activates human V γ 9V δ 2 T cells for activating human V γ 9V δ 2 T cells. Such use is for instance useful in assays such as described in the examples.

In a further embodiment, said immunoglobulin molecule that activates human V γ 9V δ 2 T cells comprises a label. These immunoglobulin molecules are in particular useful for flow

cytometry of cells expressing human V γ 9V δ 2 T cell receptor. The immunoglobulin molecule may comprise a tag, e.g. a myc tag as described in the examples or an his-tag or a fluorescent protein sequence, or it may be coupled to a suitable imaging dye. Furthermore, when coupled to e.g. magnetic beads, these immunoglobulin molecules can be used for the isolation and purification of these cells from cell suspensions including those from peripheral blood. As these immunoglobulins that activate human V γ 9V δ 2 T cells, these immunoglobulin molecules are in particular useful for selecting these cells while at the same time activating and expanding the cell population. Hence, the use is further provided of these immunoglobulin molecules for labelling and/or for selecting, and for activating human V γ 9V δ 2 T cells.

In a further aspect of the invention, an immunoglobulin molecule is provided wherein the immunoglobulin molecule is an immunoglobulin molecule that does not block activation of human V γ 9V δ 2 T cells; and does not activate human V γ 9V δ 2 T cells and wherein it is a human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, comprising a CDR1 region and a CDR2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40% sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; and wherein the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; and wherein preferably the immunoglobulin molecule is a single chain antibody. In one embodiment, the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with SEQ ID NO. 2 AISWSGGSTYYADSVKG, wherein the said amino acid sequence has a T at position 9, an A at position 12, and a V at position 15. In a further embodiment, the immunoglobulin molecule is a single domain antibody, preferably wherein the single domain antibody is a llama single chain antibody or a human single chain antibody. In a further embodiment, the single chain antibody is a single domain antibody. In further embodiments, the immunoglobulin molecule or the single chain antibody or the single domain antibody, comprises one or more of the framework regions selected from the group of amino acid sequences of SEQ ID NO. 67-70.

In one embodiment, said immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that does not block activation of nor activates human V γ 9V δ 2 T cells, comprises a CDR3 region wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of SEQ ID NO. 3, 37, 40 and 43.

In a further embodiment, said immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that does not block activation of human V γ 9V δ 2 T cells, comprises a label.

These immunoglobulin molecules are in particular useful for flow cytometric or immunohistochemical detection of cells expressing human V γ 9V δ 2 T cell receptor. The immunoglobulin molecule may comprise a tag, e.g. a myc tag as described in the examples

or an his-tag or a fluorescent protein sequence, or it may be coupled to a suitable imaging dye. Furthermore, when coupled to e.g. magnetic beads, these immunoglobulin molecules can be used for the isolation and purification of these cells from cell suspensions including those from peripheral blood. These Vy9V δ 2 T cell receptor binding immunoglobulin molecules
5 can be developed as research tools for detection in immunohistochemistry, flow-cytometry, imaging, and for magnetic purification from cell suspensions. As these do not have an effect on the human Vy9V δ 2 T cells, these immunoglobulin molecules are in particular useful for selecting these cells for further uses. Hence, the use is further provided of these immunoglobulin molecules for labelling or for selecting human Vy9V δ 2 T cells.

10 In another aspect of the invention, an immunoglobulin molecule is provided wherein the immunoglobulin molecule is an immunoglobulin comprising a CDR1 region and a CDR2 region, wherein the CDR1 region comprises an amino acid sequence with at least 40% sequence identity with the amino acid sequence of SEQ ID NO. 31 GRTFSNYAMG; and wherein the CDR2 region comprises an amino acid sequence with at least 60% sequence
15 identity with the amino acid sequence of SEQ ID NO. 32 AISWSGGSTYYADSVKG; and wherein preferably the immunoglobulin molecule is a single chain antibody. In one embodiment, the CDR2 region comprises an amino acid sequence with at least 60% sequence identity with SEQ ID NO. 2 AISWSGGSTYYADSVKG, wherein the said amino acid sequence has a T at position 9, an A at position 12, and a V at position 15. In a further
20 embodiment, the immunoglobulin molecule is a single domain antibody, preferably wherein the single domain antibody is a llama single chain antibody or a human single chain antibody. In a further embodiment, the single chain antibody is a single domain antibody. In further embodiments, the immunoglobulin molecule or the single chain antibody or the single domain antibody, comprises one or more of the framework regions selected from the group of amino
25 acid sequences of SEQ ID NO. 67-70. In a further embodiment of this aspect of the invention, the immunoglobulin molecule, the single chain antibody or the single domain antibody comprises a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 3, 6, 9, 11, 14, 17, 20, 22, 25, 27, 29, 30, 33, 35, 37, 40, 43, and 46.

30 In another aspect of the invention an immunoglobulin molecule is provided, wherein the immunoglobulin molecule comprises a CDR3 region, wherein the CDR3 region comprises an amino acid sequence selected from the group consisting of amino acid sequences SEQ ID NO. 3, 6, 9, 11, 14, 17, 20, 22, 25, 27, 29, 30, 33, 35, 37, 40, 43, and 46.

35 Examples:

Generation of donor-derived Vy9V δ 2 T cells

Healthy donor-derived (human) Vy9V δ 2 T cells were generated and cultured as described (Schneiders FL, et al. Clin Immunol 2012; 142:194-200).

Generation of Jurkat V γ 9V δ 2 T cell lines and Jurma V γ 9V δ 2 T cell lines

Jurkat and JurMa cell lines expressing V γ 9V δ 2 TCR were synthesized according to methodologies described previously (Scholten KB, et al. Clin Immunol 2006; 119:135-45). In brief, protein sequences of clone G9 V γ 9- and V δ 2-chain (Allison TJ et al. Nature 2001; 411:820-4), Davodeau F et al. Eur J Immunol 1993; 23:804-8) separated by a picorna virus derived 2A sequence was codon modified for optimal protein production and synthesized by GeneART (Life technologies) and subsequently cloned to LZRS. After transfection to the Phoenix-A packaging cell line, retroviral supernatants were collected to transduce Jurkat or Jurma cells.

Selection of anti-V γ 9V δ 2 TCR VHH

2 Llama glamas were immunized four times each with 1×10^8 human donor-derived V γ 9V δ 2 T cells in sterile PBS over a period of six weeks. Phage libraries were constructed from extracted RNA isolated from llama PBMCs as described (Roovers RC et al. Cancer Immunol Immunother. 2007;56:303-17) and ligated into phagemid vector pUR8100. V γ 9V δ 2 T cell receptor (TCR) specific VHH were generated by two rounds of phage display selections. In round 1, phages from both libraries were incubated for 2 hours at 4°C with Jurkat cells transduced to stably express V γ 9V δ 2 TCR (Jurkat V γ 9V δ 2). After incubation, cells were washed and phages were eluted with 100 mM HCl. After 7 minutes incubation at 4°C, unbound phages were removed and neutralized with Tris-HCl after which they were infected to E.coli. After recovery of selected phages, second round phages were first counter selected 2x for 1 hour at 4°C to Jurkat cells after which unbound phages were incubated for 1 hour with Jurkat V γ 9V δ 2. Phages were eluted and infected to E. coli as described for first round selections. Bacteria were plated on LB/2% glucose/ ampicillin plates to generate single bacterial colonies coding eluted VHH DNA.

Production and purification of VHH

VHH DNA from individual clones were digested with Sfi1/ BstEII and cloned into plasmid pMEK219, a derivative from pHen1 (Hoogenboom et al. Nucleic Acids Res 1991) with addition of a HC-V cassette to enable Sfi1/BstEII cloning, add a C-terminal myc-and 6x HIS-tag deletion of the genIII sequence. pMEK219-VHH was transformed to TG1 bacteria. An overnight culture was used to inoculate 2xTY medium plus 0,1% glucose and 100 ug/ml ampicillin. When OD₆₀₀ reached 0.5, IPTG was added to a final concentration of 1 mM. Protein production was allowed for 2-5 hours. Growth of all cultures was performed at 37°C with shaking at 200-220 rpm. Protein production was stopped by spinning cultures for 15 minutes at 4°C. The bacterial pellet was suspended in PBS and frozen for at least 1 hour. Bacterial suspension was thawed, slightly shaken for 1 hour at 4°C and spun at 4500 rpm for

30 minutes. Supernatant was incubated with washed Talon resin (Clontech, 1290 Terra Bella Ave. Mountain View, CA, USA) for 1 hour at room temperature. Talon resin was washed 3x with PBS and 1x with 15 mM imidazole/PBS pH7 and eluted with 150 mM imidazole/PBS pH7. The eluted fraction was dialysed 2x against PBS. Purified VHH was checked by
5 coomassie stained protein gel for purity.

Binding of VHH to donor-derived Vy9Vδ2 T cells or Jurkat Vy9Vδ2 T cells.

5*10⁴ donor-derived Vy9Vδ2 T cells were washed with FACS buffer. All incubations were performed in FACS buffer for 30 minutes at 4°C. Cells were incubated with 25 µl 500 nM
10 VHH. After washing, cells were incubated with 10 µl 1:500 anti-myc tag antibody clone 4A6 (Merck Millipore, 290 Cocord Road Billerica, MA, USA). After washing, cells were incubated with 10 µl 1:200 goat-anti-mouse F(ab)2 APC (Beckman Coulter, Fullerton, CA, USA) for 30 minutes at 4°C. After a final washing step, VHH binding to cells was measured by
flowcytometry (FACSCalibur, BD Biosciences).

Activation of donor-derived Vy9Vδ2 T cells by VHH

Flat bottom 96-well cell culture plates (Costar) were coated overnight with 50 µl 4 µg/ml mouse-anti-myc clone 9E10 (made in house) at 4°C. Wells were washed with PBS and blocked with 200 µl 4% BSA/PBS at room temperature for 30 minutes. Block was discarded
20 and wells were incubated with 30 µl 500 nM VHH in PBS for 2 hours at room temperature. Wells were washed and 1x10⁴ Vy9Vδ2 T in 200 µl IMDM+ (Schneiders FL, et al.Clin Immunol 2012; 142:194-200) were added per well and incubated overnight at 37°C in a CO₂ incubator with humidified atmosphere in the presence of golgiplug (1:500) (BD Biosciences) for intracellular cytokine retention. Flowcytometry was then used to determine CD25, IFN-γ
25 and Granzyme B expression (as described; Schneiders FL, et al.Clin Immunol 2012; 142:194-200 (CD25-PE (clone M-A251, #555432) , IFN-γ APC (clone B27 #554702) both available from BD Pharmingen. Granzyme B PE (clone GB-12 #M2289) available from Sanquin, Amsterdam, The Netherlands).

Neutralization of donor-derived Vy9Vδ2 T cells by VHH

HeLa cells were incubated with indicated amounts of aminobisphosphonates (NBP;ABP Pamidronaat-DiNatrium, Pharmachemie, Haarlem, The Netherlands) for 2 hours at 37°C in a CO₂ incubator with humidified atmosphere. Cells were then washed and seeded at 5*10⁴ in 100 µl IMDM+ per well in a flat bottom 96-well cell culture plate (Costar) and allowed to
35 adhere for 2 hours at 37°C in a CO₂ incubator with humidified atmosphere. Cells were washed with PBS and cultured in 100 µl IMDM+. Donor-derived Vy9Vδ2 T cells were incubated with the indicated VHH concentration for 1 hour at 4°C. 75*10³ VHH-incubated Vy9Vδ2 T cells were added to NBP-treated HeLa cell coated wells and incubated at 37°C in a

CO₂ incubator with humidified atmosphere. Cells were harvested with trypsin to a 96-wells round bottom plate, Golgiplug (1:500, BD Biosciences) was added for intracellular cytokine retention. Flowcytometry was used to determine CD25, IFN- γ and Granzyme B expression (as described; Schneiders FL, et al. Clin Immunol 2012; 142:194-200)

5

VHH chain specificity

A donor-derived V γ 9V δ 2 T cell line was stained with mouse-anti-human V δ 2-FITC and mouse-anti-human V γ 9-PE (both BD Biosciences) and sorted with FACS Aria (BD Biosciences) for the populations: single V δ 2 positive $\gamma\delta$ T-cells, single V γ 9 positive $\gamma\delta$ T-cells, 10 V γ 9V δ 2 double positive $\gamma\delta$ T-cells and V γ 9V δ 2 double negative $\gamma\delta$ T-cells. Sorted cells were cultured in the same way as the donor-derived V γ 9V δ 2 T cell lines. For determining VHH specificity, 10⁴ cells of the resulting purified sorted cell lines were stained with VHH similar to the methodology as described for binding of VHH to donor-derived V γ 9V δ 2 T cells with the adjustment that 10 μ l 1:80 goat-anti-mouse-F(ab)₂ RPE (#R0480 from Dako, Glostrup, 15 Denmark) was used for anti-myc antibody detection.

Results

The selected VHHs were tested for specificity as described above, and all 20 VHHs (see table 2) showed binding to V γ 9V δ 2 T cell receptor expressing Jurkat cells as well as primary 20 V γ 9V δ 2 T cells, whereas they did not bind to Jurkat cells not expressing the V γ 9V δ 2 T cell receptor.

Immunoglobulin molecules that block phosphoantigen induced activation

Clones 6F6 and 5E7 were characterized as nanobodies that block phosphoantigen-induced 25 stimulation of V γ 9V δ 2 T cells. Both clones 6F6 and 5E7 are nanobodies that bind to the V δ 2 chain of the V γ 9V δ 2 T cell receptor. GrB, CD25 and IFN- γ expression were similar to unstimulated controls, whereas the positive control showed relative high expression levels (see figure 2). In a dose response curve, upon exposure to phosphoantigen, dose dependent neutralization of phosphoantigen induced V γ 9V δ 2 T cell activation was shown (see figure 3). It 30 was further shown that the VHH 5E7 nanobody inhibits V γ 9V δ 2 T cell activation by aminobisphosphonates (ABP) in a dose dependent manner, i.e. a higher dose of 5E7 results in a relative stronger reduction of CD25 and CD107a expression, and a relative stronger reduction of interferon- γ and TNF- α production as well. The 5E7 nanobody was also shown to inhibit spontaneous lysis of Daudi cells by V γ 9V δ 2 T cells in a dose dependent manner, 35 whereas a control nanobody did not show any significant effect. In the same assay, the nitrogen-containing bisphosphonate pamidronate was used to activate V γ 9V δ 2 T cells resulting in an enhanced lysis of Daudi cells. Again, the 5E7 nanobody reduced the lysis of the Daudi cells in a dose dependent manner. This indicates that any undesired activation of

V γ 9V δ 2 T cells may be reduced by using a nanobody that blocks V γ 9V δ 2 T cell activation. Such an antibody that blocks V γ 9V δ 2 T cell activation may be an antibody that binds to the V δ 2 chain of the V γ 9V δ 2 T cell receptor.

5 *Immunoglobulin molecules that induce activation*

Various VHHs were shown to activate V γ 9V δ 2 T cells as shown by an increase in CD25 expression and an increase in IFN-gamma expression (see figure 4). Furthermore, such VHHs showed a typical dose response as an increasing dose of VHHs resulted in an increasing CD25 expression as well (see figure 5, right panel). Such a VHH was also coupled
 10 to an immunoglobulin molecule and the effect on apoptosis of tumour cells studied (see figure 6). The bispecific VHH (anticancer cell binding and V γ 9V δ 2 T cell binding and activation) showed potent activity towards killing of tumour cells. A bispecific VHH was made by coupling of anti-V γ 9V δ 2 nanobody 6H4 to a nanobody against a tumor. As bispecific controls, an anti-V γ 9V δ 2 nanobody was coupled to a control nanobody, and an anti-tumor nanobody
 15 was coupled to a control nanobody. At the highest dose tested (10nM), the controls only induced about 22% lysis of tumor cells. The bispecific VHH (or nanobody) binding both V γ 9V δ 2 T cells and tumor cells induced about 85% lysis of the tumor cells mediated by the V γ 9V δ 2 T cells. In a dose response curve, the percentage of lysis by the V γ 9V δ 2 T cells decreased with a lower dose (1nM, about 80%, 100pM about 78%, 10 pM about 50%, 1 pM
 20 about 23% and 0 about 24%). In a control experiment without (bispecific) nanobodies only using bisphosphonates, about 80% of tumor cell lysis was observed. These results show that tumor-specific lysis by V γ 9V δ 2 T cells can be enhanced by using bispecific VHHs (or nanobodies), wherein both the tumor and V γ 9V δ 2 T cells are targeted, and wherein the specific tumor targeting of V γ 9V δ 2 T cells induces activation of V γ 9V δ 2 T cells as well.

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Immunoglobulin molecules that do not induce activation and do not block phosphoantigen activation

Several VHHs (5D7, 5C7, 5B11 and 6C4) showed no activation of human V γ 9V δ 2 T cells, nor did it have an effect on blocking phosphoantigen human V γ 9V δ 2 T cell activation (figure 8
 30 and figure 5, left panel). Such VHHs are useful for example in FACS sorting (see figure 7).

Magnetic bead separation

An anti-V δ 2 (e.g. 6H4) or V γ 9 nanobody (e.g. 6H1) was biotinylated and mixed with PBMCs. The cells were washed to remove unbound nanobody. Magnetic beads with streptavidin
 35 (such as available from Miltenyi Biotec) were added to the mixture and cells bound to the beads, via the biotinylated nanobody, separated from unbound cells using a magnetic separating column. PBMCs were FACS analysed with regard to V γ 9 and V δ 2 expression. Excellent purification was obtained with both anti-V δ 2 and V γ 9 nanobodies. For example, with

nanobody 6H4 4.5% of the PBMCs expressed both chains, after magnetic bead separation, 97,4% of the cells were positive for both V γ 9 and V δ 2 chains. The fraction of cells that did not bind to the magnetic beads were negative for both V γ 9 and V δ 2 chains (0%).

- 5 Table 2. Binding of VHHs to $\gamma\delta$ T- cells expressing V γ 9V δ 2 or not expressing V γ 9V δ 2 or expressing a single V γ 9 or V δ 2 chain.

Nr	Ref	V δ 2 +	V γ 9 +	V γ 9V δ 2 +	V γ 9V δ 2 -
1	5C7	+/-	-	+/-	-
2	5E3	-	++	++	-
3	6H1	-	++	++	-
4	5G3	-	++	++	-
5	5C1	+/-	++	++	-
6	5D3	++	-	++	-
7	6E3	++	-	++	-
8	6H4	++	-	++	-
9	6C1	++	-	++	-
10	6H3	++	+/-	++	-
11	6G3	++	-	++	-
12	6F6	++	-	++	-
13	5C8	++	-	++	-
14	5E7	++	-	++	-
15	5F5	++	-	++	-
16	6A1	++	-	++	-
17	5D7	++	-	++	-
18	5B11	-	-	+	-
19	6C4	+/-	++	++	-
20	6E4	++	-	++	-

Table 3. Sequences. (B= binding, not activating, not phosphoantigen activation (PA) blocking; A= activating; PA= blocks PA activation)

10

SE Q ID.	code	Description		Sequence
1	5C7	CDR1	B	GRTFSRYTMG
2	5C7	CDR2	B	AISWSSGRTNFAFSVKG

3	5C7	CDR3	B	DWLPVPGRESYDY
4	5E3	CDR1	A	GRTFSSYAMG
5	5E3	CDR2	A	AISWSGGTTYADSVKG
6	5E3	CDR3	A	SLDCSGPGCHTAEYDY
7	6H1	CDR1	A	GRTFSEYAMG
8	6H1	CDR2	A	AISWTGSKTTYADSVKG
9	6H1	CDR3	A	SSDCSGPGCHTEEYDY
4	5G3	CDR1	A	GRTFSSYAMG
10	5G3	CDR2	A	AVSWSGGSTYYADSVKG
11	5G3	CDR3	A	SQDCSGPGCYTNEYDS
12	5C1	CDR1	A	GSIFSNYAMA
13	5C1	CDR2	A	AVSWSGGRTYYADSVKG
14	5C1	CDR3	A	SLSCSGPGCSLEEYDY
15	5D3	CDR1	A	GRPFSNYAMG
16	5D3	CDR2	A	VISWSGGSTYYADSVKG
17	5D3	CDR3	A	QFSGASTVVAGTALDYDY
18	6E3	CDR1	A	GRPFSNYGMG
19	6E3	CDR2	A	GISWSGGSTDYADSVKG
20	6E3	CDR3	A	VFSGAETAYYPSDDYDY
18	6H4	CDR1	A	GRPFSNYGMG
19	6H4	CDR2	A	GISWSGGSTDYADSVKG
20	6H4	CDR3	A	VFSGAETAYYPSDDYDY
18	6C1	CDR1	A	GRPFSNYGMG
19	6C1	CDR2	A	GISWSGGSTDYADSVKG
20	6C1	CDR3	A	VFSGAETAYYPSDDYDY
18	6H3	CDR1	A	GRPFSNYGMG
21	6H3	CDR2	A	GITWSGGSTHYADLVKG
22	6H3	CDR3	A	VFSGAETAYYPSTEYDY
23	6G3	CDR1	A	GRPFNNYGMG
24	6G3	CDR2	A	GISWSGGSTYYADSVKG
25	6G3	CDR3	A	VFSGAETAQYPSYDYDY
15	6F6	CDR1	PA	GRPFSNYAMG
26	6F6	CDR2	PA	AVTWSGGSTYYADSVKG
27	6F6	CDR3	PA	QFNGAENIVPATTTPTS YDY
15	5C8	CDR1	A	GRPFSNYAMG
28	5C8	CDR2	A	AISWSGGSTSYADSVKG

29	5C8	CDR3	A	QFSGADYGFGRLGIRGYEYDY
15	5E7	CDR1	PA	GRPFSNYAMG
28	5E7	CDR2	PA	AISWSGGSTSYADSVKG
30	5E7	CDR3	PA	QFSGADYGFGRLGIQGYEYDY
31	5F5	CDR1	A	GRTFSNYAMG
32	5F5	CDR2	A	AISWSGGSTYYADSVKG
33	5F5	CDR3	A	MFSGSESQLVVTNLYEYDY
31	6A1	CDR1	A	GRTFSNYAMG
34	6A1	CDR2	A	TISWSGGSTYYADSVKG
35	6A1	CDR3	A	AFSGSDYANTKKEVEYDY
31	5D7	CDR1	B	GRTFSNYAMG
36	5D7	CDR2	B	AISWSGGMTDHADSVKG
37	5D7	CDR3	B	AFAGDIPYGSSWYGDPTTYDY
38	5B11	CDR1	B	GRTSSTFSMA
39	5B11	CDR2	B	AINWSGGSTRYADSVSD
40	5B11	CDR3	B	RRGGIYYSTQNDYDY
41	6C4	CDR1	B	VRTFSDYRMG
42	6C4	CDR2	B	TISWSGGLTYADSVKG
43	6C4	CDR3	B	GGGYAGGTYYHPEE
44	6E4	CDR1	A	GFTFDDYCIA
45	6E4	CDR2	A	CITTSDGSTYYADSVKG
46	6E4	CDR3	A	YFGYGCYGAQDYRAMDY
47	5C7	VHH		EVQLVESGGGLVQAGDSLRLSCAASGRTFSRYTMGWF RQAPGKEREFVAAISWSGGRTNFAGSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAADWLPVPGRESYDY WGQGTQVTVSS
48	5E3	VHH		EVQLVESGGGLVQAGGSLRLSCTASGRTFSSYAMGWF RQAPGKEREFVAAISWSGGTTYADSVKGRFTISRDN AKNTVSLQMNSLKPEDTAVYFCAASLDCSGPGCHTAE YDYWGQGTQVTVSS
49	6H1	VHH		EVQLVESGGGLVQAGGSLRLSCAATGRTFSEYAMGWF RQAPGKEREFAAAISWTGSKTYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAASSDCSGPGCHTEE YDYWGQGTQVTVSS
50	5G3	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRTFSSYAMGWF RQAPGKEREFVAAVSWSGGSTYYADSVKGRFTISRDN ARNTVYLQMNSLNPEDTAVYYCAASQDCSGPGCYTNE

				YDSWGQGTQVTVSS
51	5C1	VHH		EVQLVESGGGLVQPGGSLRLSCAASGSIFSNYAMAWF RQAPEKERDFLAAVSWSGGRITYYADSVKGRFTISRDN AKNTVNLQMNSLKPEDTAVYYCAASLSCSGPGCSLEE YDYWGQGTQVTVSS
52	5D3	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFPSNYAMGWF RQAPGKEREFVTVISWSGGSTYYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAAQFSGASTVVAGTA LDYDYWGQGTRVTVSS
53	6E3	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFPSNYGMGWF RQAPGKKREFVAGISWSGGSTDYADSVKGRLTISRDN AKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSD DYDYWGQGTQVTVSS
54	6H4	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFPSNYGMGWF RQAPGKKREFVAGISWSGGSTDYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSD DYDYWGQGTQVTVSS
55	6C1	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFPSNYGMGWF RQAPGKKRESVAGISWSGGSTDYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAYYPSD DYDYWGQGTQVTVSS
56	6H3	VHH		EVQLVESGGGLVQAGGSLRLSCAVSGRPFPSNYGMGWF RQAPGKEREFVAGITWSGGSTHYADLVKGRFTISRDN AKNTVHLQMNSLKPEDTAVYYCAAVFSGAETAYYPSY EYDYWGQGTQVTVSS
57	6G3	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFNNYGMGWF RQAPGKEREFVAGISWSGGSTYYADSVKGRFTISRDN AKNTVYLQMNSLKPEDTAVYYCAAVFSGAETAQYPSY DYDYWGQGTQVTVSS
58	6F6	VHH		EVQLVESGGGLVQAGGSLRLSCVASGRPFPSNYAMGWF RQAPGKEREFVAAVTWSGGSTYYADSVKGRFAISRDN AKNTVYLQMNSLKPEDTAVYYCAAQFNGAENIVPATT TPTSYDYWGQGTQVTVSS
59	5C8	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFPSNYAMGWF RQAPGKEREFVAAISWSGGSTSYADSVKGRFTISRDN AKNTVYLQMNSPKPEDTAIYYCAAQFSGADYGFGRLLG IRGYEYDYWGQGTQVTVSS
60	5E7	VHH		EVQLVESGGGLVQAGGSLRLSCAASGRPFPSNYAMGWF

				RQAPGKERE FVAAISWSGGSTSYADSVKGRFTISRDN AENTVY LQMNSPKPEDTAIYYCAAQFSGADYGFGR LG IQGYEYDYWGQGTQVTVSS
61	5F5	VHH		EVQLVESGGGLVQAGGSLRLS CAASGRTFSNYAMGWF RQAPGKERE FVAAISWSGGSTYYADSVKGRFTISRDN AKNTVY LQMNSLKPEDTAVYYCAAMFSGSESQ LVVVI TNLYEYDYWGQGTQVTVSS
62	6A1	VHH		EVQLVESGGGLVQAGGSLRLS CAASGRTFSNYAMGWF RQAPGKERE FVATISWSGGSTYYADSVKGRFTISRDN AKNTVY LQMNSLKPEDTAVYYCAA FSGSDYANTKKE VEYDYWGQGTQVTVSS
63	5D7	VHH		1EVQLVESGGGLVQAGGSLRLS CIA SGRTFSNYAMGW FRQAPGKERE FVAAISWSGGMTDHADSVKGRFTISRDN NAKNTVY LQMNSLKPEDTAVYYCAA FAGDIPYGSSW YGDPTTYDYWGQGTQVTVSS
64	B11	VHH		EVQLVESGGGLVQAGGSLRLS CAASGRTSSTFSMAWF RQAPRKERE FVAAINWSGGSTRYADSVSDRFAISRDN AKNTVY LQMNNLKPEDTAVYYCAARRGGIYYSTQNDY DYWGQGTQVTVSS
65	6C4	VHH		3EVQLVESGGGLVQAGGSLRLS CAVSVRTFSDYRMGW FRQAPGKERE FVSTISWSGGLTYADSVKGRFTISRDN NSKNTLY LQMNSLKPEDTAVYYCAAGGGYAGGTYYPH EEWGQGTQVTVSS
66	6E4	VHH		EVQLVESGGGLVQAGGSLRLS CAASGFTFDDYCIAWF RQAPGKERE PVSCITTS DGSTYYADSVKGRFTISSDN AKNTVY LQMNLKPEDTAVYYCAAYFGYGCYGAQDY RAMDYWGKGT LVTVSS
67	5C7	FRW1		EVQLVESGGGLVQAGDSLRLS CAAS
68	5C7	FRW2		WFRQAPGKERE FVA
69	5C7	FRW3		RFTISRDN AKNTVY LQMNSLKPEDTAVYYCAA
70	5C7	FRW4		WGQGTQVTVSS
71	Human V gamma 9 chain	TCR		MLSL LHASTLAVLGALCVYGAGHLEQPQISSTKTL SK TARLECVVSGITISATSVYWYRERPGEVIQFLVSISY DGTVRKESGIPSGKFEVD RIPETSTSTLT IHNVEKQD IATYYCALWEAQQELGKKIKVFGPGTKLIITDKQLDA DVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIK IHWEEKKSNTILGSQEGNTMKTNDTYMKFSWLTVPEK

				SLDKEHRCIVRHENNKNGVDQEIIFFPIKTDVITMDP KDNCSKDANDTLLLQLTNTSAYMYLLLLLLKSVVYFA IITCCLLRRTAFCCNGEKS
72	Human V delta 2 chain	TCR		MQRISSLIHLSLFWAGVMSAIELVPEHQTVPVSIGVP ATLRCSMKGEAIGNYYINWYRKTQGNTMTFIYREKDI YGPFGKDNFQGDIDIAKNLAVLKILAPSERDEGSYYC ACDTLGMGGEYTDKLIFGKGTRVTVEPRSQPHTKPSV FVMKNGTNVACLVKEFYPKDIRINLVSSKKITEFDPA IVISPSGKYNAVKLGKYEDSNSVTCSVQHDNKT VHST DFEVKTDSTDHVKPKETENTKQPSKSKCHKPKAIVHTE KVNMMSLTVLGLRMLFAKTVAVNFLLTAKLFFL

CLAIMS

1. A human V γ 9V δ 2 T cell receptor binding immunoglobulin molecule, comprising a CDR1 region, a CDR 2 region, and a CDR 3 region, wherein the CDR1, CDR2 and CDR3 regions comprise:
- the amino acid sequences set forth in SEQ ID Nos: 4, 5 and 6, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 7, 8 and 9, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 4, 10 and 11, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 12, 13 and 14, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 15, 16 and 17, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 18, 19 and 20, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 18, 21 and 22, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 23, 24 and 25, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 15, 28 and 29, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 31, 32 and 33, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 31, 34 and 35, respectively, or
 - the amino acid sequences set forth in SEQ ID Nos: 44, 45 and 46, respectively,
- wherein the immunoglobulin molecule is an immunoglobulin molecule that activates human V γ 9V δ 2 T cells.
2. The immunoglobulin molecule according to claim 1, wherein the immunoglobulin molecule is a single chain antibody, preferably wherein the single chain antibody is a llama single chain antibody or a human single chain antibody.
3. The immunoglobulin molecule according to claim 1, wherein the immunoglobulin molecule is a single domain antibody, preferably wherein the single domain antibody is a llama single domain antibody or a human single domain antibody.
4. The immunoglobulin molecule according to any one of claims 1-3, comprising one or more of the framework regions selected from the group of amino acid sequences of SEQ ID NO. 67-70.
5. A nucleotide sequence that encodes an immunoglobulin molecule according to any one of claims 1-4.
6. A host cell comprising a nucleotide sequence according to claim 5.

7. Method for preparing an immunoglobulin molecule according to any one of claims 1-4, comprising:
- culturing a host cell according claim 6;
 - allowing the host cell to express the immunoglobulin;
 - obtaining the immunoglobulin.
8. An immunoglobulin molecule according to any one of claims 1-4 for use in a medical treatment.
9. The immunoglobulin molecule according to any one of claims 1-4, wherein the immunoglobulin molecule is linked to an agent.
10. An immunoglobulin molecule according to any one of claims 1-4, or 9 for use in a medical treatment, wherein preferably the medical treatment is a treatment of cancer.
11. Non-therapeutic use of an immunoglobulin molecule according to any one of claims 1-4, or 9 for activating human V γ 9V δ 2 T cells.
12. An immunoglobulin molecule according to any one of claims 1-4, or 9 for activating human V γ 9V δ 2 T cells.
13. The immunoglobulin molecule according to claim 1, which is a bispecific antibody comprising two single domain antibodies, the first single domain antibody comprising the CDR1, CDR2 and CDR3 regions as defined in claim 1, wherein the first single domain antibody is linked to a second single domain antibody wherein the second single domain antibody binds to a target.
14. The immunoglobulin molecule according to claim 1, which is a bispecific antibody comprising two single domain antibodies, the first single domain antibody comprising the CDR1, CDR2 and CDR3 regions as defined in claim 1, wherein the first single domain antibody is linked to a second single domain antibody wherein the second single domain antibody binds to a cancer cell.