

Title: Immunoglobulins binding human V $\gamma$ 9V $\delta$ 2 T cell receptors

5 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 $\gamma$ 9V $\delta$ 2 T cell receptors. The invention provides for immunoglobulin molecules that bind a human V $\gamma$ 9V $\delta$ 2 T cell receptor, such as antibodies, single chain antibodies, or single domain antibodies, wherein the human V $\gamma$ 9V $\delta$ 2  
10 T cells may be modulated.

Background: The majority of  $\gamma\delta$  peripheral blood lymphocytes (PBLs) in human adults express T-cell receptors (TCRs) comprising V $\gamma$ 9 and V $\delta$ 2 regions. V $\gamma$ 9V $\delta$ 2 T cells can react against a wide array of pathogens and tumour cells. This broad reactivity is understood to be  
15 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 $\gamma$ 9V $\delta$ 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 $\gamma$ 9V $\delta$ 2 T cells may be overstimulated or inadvertently activated.

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

30 Summary: The current disclosure now provides for novel agents that can bind to V $\gamma$ 9V $\delta$ 2 T cells. The agents provided are immunoglobulins. The immunoglobulins provided bind to a V $\gamma$ 9V $\delta$ 2 T cell receptor. Surprisingly, it was found that the immunoglobulins provided by the current disclosure have a substantial sequence identity. Hence, in a first example of the  
35 disclosure, human V $\gamma$ 9V $\delta$ 2 T cell receptor binding immunoglobulin molecules are 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; 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.

5 In one aspect, the present disclosure refers to a bispecific immunoglobulin molecule comprising an antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells linked to a tumor antigen-specific antibody, wherein said antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells binds the V $\delta$ 2 chain of a V $\gamma$ 9V $\delta$ 2 T cell receptor, and wherein the tumor antigen-specific antibody has one antigen-binding site.

10 Furthermore, such immunoglobulins comprise a CDR3 region, wherein the CDR3 region contributes to V $\gamma$ 9V $\delta$ 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 $\gamma$ 9V $\delta$ 2 T cells, inducing activation of V $\gamma$ 9V $\delta$ 2 T cells or neither blocking activation nor inducing activation of V $\gamma$ 9V $\delta$ 2 T cells. The immunoglobulins of the  
15 disclosure are in particular for use in medical treatments and for use in assays involving V $\gamma$ 9V $\delta$ 2 T cells.

In one aspect, the present disclosure refers to use of a bispecific immunoglobulin molecule disclosed herein in the manufacture of a medicament for the treatment of cancer.

20 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, 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.

## 25 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 indicated as well (i.e. 5C7 is the sequence of VHH 5C7).

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

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

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

## CLAIMS

1. A bispecific immunoglobulin molecule comprising an antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells linked to a tumor antigen-specific antibody, wherein said antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells binds the V $\delta$ 2 chain of a V $\gamma$ 9V $\delta$ 2 T cell receptor, and wherein the tumor antigen-specific antibody has one antigen-binding site.  
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2. The bispecific immunoglobulin molecule according to claim 1, wherein said antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells is a single chain or a single domain antibody.  
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3. The bispecific immunoglobulin molecule according to claim 1 or 2, wherein said antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells is a single chain antibody and said tumor antigen-specific antibody is a single chain antibody.
4. The bispecific immunoglobulin molecule according to claim 1 or 2, wherein said antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells is a single domain antibody and said tumor antigen-specific antibody is a single domain antibody.  
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5. The bispecific immunoglobulin molecule according to any one of the preceding claims, wherein said antibody that activates human V $\gamma$ 9V $\delta$ 2 T cells binds V $\gamma$ 9V $\delta$ 2 double-positive T cells and V $\delta$ 2 single-positive T cells, but not V $\gamma$ 9 single-positive T cells.  
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6. Use of a bispecific immunoglobulin molecule according to any one of claims 1 to 5 in the manufacture of a medicament for the treatment of cancer.