Title: Immunoglobulins binding human Vγ9Vδ2 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 Vγ9Vδ2 T cell receptors. The invention provides for immunoglobulin molecules that bind a human Vγ9Vδ2 T cell receptor, such as antibodies, single chain antibodies, or single domain antibodies, wherein the human Vγ9Vδ2 T cells may be modulated.

Background: The majority of γδ peripheral blood lymphocytes (PBLs) in human adults express T-cell receptors (TCRs) comprising Vγ9 and Vδ2 regions. Vγ9Vδ2 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 Vγ9Vδ2 T-cells suggest a direct involvement in immune control of cancers and infections. In addition to fighting disease, in some diseases or medical treatment Vγ9Vδ2 T cells may be overstimulated or inadvertently activated.

Hence, agents that can activate Vγ9Vδ2 T cells can be useful in the treatment of infections or cancer as these may promote Vγ9Vδ2 T cell reactivity towards the pathogen or infected cells or cancer. Furthermore, agents that block activation of Vγ9Vδ2 T cells may be useful in diseases or medical treatment where it is advantageous to reduce Vγ9Vδ2 T cell activation, i.e. wherein Vγ9Vδ2 T cells are overstimulated or inadvertently activated. Finally, agents that can bind a Vγ9Vδ2 T cell, but do not have an effect on (phosphoantigen) activation of Vγ9Vδ2 T cells are useful for labelling cells, for example for selecting or identifying Vγ9Vδ2 T cells. Hence, there is a need in the art to provide for agents that can bind to Vγ9Vδ2 T cells, and for agents that can block phosphoantigen activation of Vγ9Vδ2 T cells or can activate Vγ9Vδ2 T cells.

Summary ~~of the invention~~: The current ~~invention~~disclosure now provides for novel agents that can bind to Vγ9Vδ2 T cells. The agents provided are immunoglobulins. The immunoglobulins provided bind to a Vγ9Vδ2 T cell receptor. Surprisingly, it was found that the immunoglobulins provided by the current ~~invention~~disclosure have a substantial sequence identity. Hence, in a first ~~aspect~~example of the ~~invention~~disclosure, human Vγ9Vδ2 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.

In one aspect, the present disclosure refers to a bispecific immunoglobulin molecule comprising an antibody that activates human Vγ9Vδ2 T cells linked to a tumor antigen-specific antibody, wherein said antibody that activates human Vγ9Vδ2 T cells binds the Vδ2 chain of a Vγ9Vδ2 T cell receptor, and wherein the tumor antigen-specific antibody has one antigen-binding site.

Furthermore, such immunoglobulins comprise a CDR3 region, wherein the CDR3 region contributes to Vγ9Vδ2 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 Vγ9Vδ2 T cells, inducing activation of Vγ9Vδ2 T cells or neither blocking activation nor inducing activation of Vγ9Vδ2 T cells. The immunoglobulins of the ~~invention~~disclosure are in particular for use in medical treatments and for use in assays involving Vγ9Vδ2 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.

Preferably, the immunoglobulin molecules according to the ~~invention~~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.

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 Vγ9Vδ2 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 Vγ9Vδ2 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 Vγ9Vδ2 T cells. A representative example demonstrates the dose dependent neutralization of phosphoAg-induced Vγ9Vδ2 T cell activation using VHH 5E7 while a non-specific VHH (negative control) cannot neutralize phosphoAg-induced Vγ9Vδ2 T cell activation. Vertical axis indicates activation of Vγ9Vδ2 T cells as assessed by CD25 expression, horizontal axis indicates different VHH concentrations. Vγ9Vδ2 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: Vγ9Vδ2 TCR specific VHH are capable of inducing activation and cytokine production in healthy donor-derived Vγ9Vδ2 T cells. Data indicate relative expression of the activation marker CD25 and the pro-inflammatory cytokine IFN-γ by healthy donor-derived Vγ9Vδ2 T cells in comparison with the positive control (phosphoantigen (pAg+) expressing

CLAIMS

1. A human Vγ9Vδ2 T cell receptor binding immunoglobulin molecule, 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 single chain antibody.

2. The immunoglobulin molecule according to claim 1, wherein 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.

3. The immunoglobulin molecule according to claim 1 or claim 2,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.

4. The immunoglobulin molecule according to claim 1 or claim 2, 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.

5. The immunoglobulin molecule according to any one of claims 1-4,comprising one or more of the framework regions selected from the group of amino acid sequences of SEQ ID NO. 67-70.

6. The immunoglobulin molecule according to any one of claims 1-5 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. 3, 6, 9, 11, 14, 17, 20, 22, 25, 27, 29, 30, 33, 35, 37, 40, 43, and 46.

7. The immunoglobulin molecule according to any one of claims 1-6 having the combinations of the amino acid sequences of the CDR1, CDR2 and CDR3 regions from the antibodies as listed in table 1.

~~8. The immunoglobulin molecule according to any one of claims 1-7, wherein the immunoglobulin molecule has an amino acid sequence selected from the group of amino acid sequences consisting of SEQ ID NO. 47-66.~~

~~9. A nucleotide sequence that encodes an immunoglobulin molecule according to any one of claims 1-8.~~

~~10. A host cell comprising a nucleotide sequence according to claim 9.~~

~~11. Method for preparing an immunoglobulin molecule according to any one of claims 1-8, comprising:~~
~~- culturing a host cell according claim 10;~~
~~- allowing the host cell to express the immunoglobulin;~~
~~- obtaining the immunoglobulin.~~

~~12. An immunoglobulin molecule according to any one of claims 1-8 for use in a medical treatment.~~

~~13. A human Vγ9Vδ2 T cell receptor binding immunoglobulin molecule, wherein the immunoglobulin molecule is an immunoglobulin molecule that blocks activation of human Vγ9Vδ2 T cells.~~

~~14. The immunoglobulin molecule according to claim 13, wherein the immunoglobulin molecule is an immunoglobulin molecule according to any one of claims 1-6.~~

~~15. The immunoglobulin molecule according to claim 13-14, 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. 27 and 30.~~

~~16. An immunoglobulin molecule according to any one of claims 13-15 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.~~

~~17. Use of a~~An immunoglobulin molecule according to any one of claims 13-15 for ~~blocking activation of human Vγ9Vδ2 T cells.~~

~~18. An immunoglobulin molecule according to any one of claims 1-5, wherein the immunoglobulin molecule is an immunoglobulin molecule that activates human Vγ9Vδ2 T cells.~~

~~19. The immunoglobulin molecule according to claim 18, 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. 6, 9, 11, 14, 17, 20, 22, 25, 29, 33, 35, and 46.~~

~~20. The immunoglobulin molecule according to claim 18 or claim 19, wherein the immunoglobulin molecule is linked to an agent.~~

~~21. An immunoglobulin molecule according to any one of claims 18-20 for use in a medical treatment, wherein preferably the medical treatment is a treatment of cancer.~~

~~22. Use of a~~An immunoglobulin molecule ~~according to any one of claims 18-20 for activating human Vγ9Vδ2 T cells.~~

~~23. An immunoglobulin molecule according to any one of claims 1-5, wherein the immunoglobulin molecule is an immunoglobulin molecule that does not block activation of human Vγ9Vδ2 T cells; and does not activate human Vγ9Vδ2 T cells.~~

~~24. The immunoglobulin molecule according to claim 23 wherein the immunoglobulin molecule 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.~~

~~25. The immunoglobulin molecule according to claim 23 or claim 24 wherein the immunoglobulin molecule or the antigen-binding part thereof comprises a label.~~

~~26. Use of an immunoglobulin molecule according to any one of claims 23-25 for labelling or for selecting human Vγ9Vδ2 T cells.~~

1. A bispecific immunoglobulin molecule comprising an antibody that activates human Vγ9Vδ2 T cells linked to a tumor antigen-specific antibody, wherein said antibody that activates human Vγ9Vδ2 T cells binds the Vδ2 chain of a Vγ9Vδ2 T

cell receptor, and wherein the tumor antigen-specific antibody has one antigen-binding site.

2. The bispecific immunoglobulin molecule according to claim 1, wherein said antibody that activates human Vγ9Vδ2 T cells is a single chain or a single domain antibody.

3. The bispecific immunoglobulin molecule according to claim 1 or 2, wherein said antibody that activates human Vγ9Vδ2 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 Vγ9Vδ2 T cells is a single domain antibody and said tumor antigen-specific antibody is a single domain antibody.

5. The bispecific immunoglobulin molecule according to any one of the preceding claims, wherein said antibody that activates human Vγ9Vδ2 T cells binds Vγ9Vδ2 double-positive T cells and Vδ2 single-positive T cells, but not Vγ9 single-positive T cells.

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.