

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.

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.