

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 a single-domain antibody (VHH) that activates human V γ 9V δ 2 T cells and binds to a V δ 2 chain; wherein the single-domain antibody comprises a complementarity determining region (CDR)1, a CDR2, and a CDR3, wherein the CDR1 sequence comprises SEQ ID NO: 18, the CDR2 sequence comprises SEQ ID NO: 19, and the CDR3 sequence comprises SEQ ID NO: 20; and wherein the bispecific immunoglobulin molecule further comprises a tumor antigen-specific antibody.

Furthermore, such immunoglobulins comprise a CDR3 region, wherein the CDR3 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 disclosure are in particular for use in medical treatments and for use in assays involving V γ 9V δ 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.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising the bispecific molecule disclosed herein, for the use disclosed herein.

In one aspect, the present disclosure refers to a nucleotide that encodes the bispecific immunoglobulin molecule disclosed herein.

In one aspect, the present disclosure refers to a host cell comprising the bispecific immunoglobulin molecule disclosed herein.

In one aspect, the present disclosure refers to a method for preparing the bispecific immunoglobulin molecule disclosed herein; comprising a. culturing a host cell disclosed herein, b. allowing the host cell to express the bispecific immunoglobulin; and c. obtaining the bispecific immunoglobulin.

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.

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 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)

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 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 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

CLAIMS

1. A bispecific immunoglobulin molecule comprising a single-domain antibody (VHH) that activates human V γ 9V δ 2 T cells and binds to a V δ 2 chain;
 5 wherein the single-domain antibody comprises a complementarity determining region (CDR)1, a CDR2, and a CDR3, wherein the CDR1 sequence comprises SEQ ID NO: 18, the CDR2 sequence comprises SEQ ID NO: 19, and the CDR3 sequence comprises SEQ ID NO: 20; and
 10 wherein the bispecific immunoglobulin molecule further comprises a tumor antigen-specific antibody.
2. The bispecific immunoglobulin molecule according to claim 1, wherein said single-domain antibody that activates human V γ 9V δ 2 T cells and binds to the V δ 2 comprises SEQ ID NO: 54.
3. The bispecific immunoglobulin molecule according to claim 1 or 2, wherein said
 15 single domain antibody that activates human V γ 9V δ 2 T cells is comprised in a single chain antibody.
4. The bispecific immunoglobulin molecule according to any one of claims 1-3, wherein the tumor antigen-specific antibody is a single-domain antibody (VHH).
5. The bispecific immunoglobulin molecule according to any one of claims 1-4,
 20 wherein the single-domain antibody that activates human V γ 9V δ 2 T cells binds V γ 9V δ 2 double-positive T cells and V δ 2 single-positive T cells, but not V γ 9 single-positive T cells.
6. The bispecific immunoglobulin molecule according to any one of claims 1-5,
 25 wherein the tumor antigen-specific single-domain antibody is comprised in a single chain antibody.
7. The bispecific immunoglobulin molecule according to any one of claim 1-6, comprising:
 - a. a single chain antibody comprising a CDR1 sequence comprising SEQ ID NO: 18, a CDR2 sequence comprising SEQ ID NO: 19, and a CDR3
 30 sequence comprising SEQ ID NO: 20; and
 - b. a tumor-antigen-specific antibody that is comprised in a single chain antibody.
8. The bispecific immunoglobulin molecule according to claim 7, wherein each of the single chain antibodies (a) and (b) is of a IgG1 isotype.
9. Use of a bispecific immunoglobulin molecule according to any one of claims 1 to 8
 35 in the manufacture of a medicament for the treatment of cancer.
10. A pharmaceutical composition comprising the bispecific molecule of any one of claims 1 to 8, for the use of claim 9.

11. A nucleotide that encodes the bispecific immunoglobulin molecule according to any one of claims 1-8.
12. A host cell comprising the bispecific immunoglobulin molecule according to any one of claims 1-8.
- 5 13. A method for preparing the bispecific immunoglobulin molecule according to any one of claims 1-8; comprising
 - a. culturing a host cell according to claim 10;
 - b. allowing the host cell to express the bispecific immunoglobulin; and
 - c. obtaining the bispecific immunoglobulin.