

interaction between CAR-modified T cell and tumor cell, the ensuing CAR-T cell stimulation and anti-tumor response may also be suboptimal^{3, 7}. To increase the chance of achieving a more optimized CAR-target molecule interaction, the inventors have previously investigated variants of IgG4-derived spacers that differ in length and composition. The paradigm that emerged was that there is a correlation between spacer length and efficacy whereby membrane-proximal epitopes on target cells are reached better by CARs containing longer spacer, and membrane-distal epitopes by CARs containing a shorter one⁷. Based on the architecture of the IgG4 molecule, three IgG4-Hinge based spacer variants are available that differ in size in increments > 100 aa (IgG4_short: IgG4 Hinge, 12 aa; IgG4_intermediate: IgG4 Hinge+C_H3, 119 aa; IgG4_long: IgG4 Hinge+CH₂+CH₃, 228 aa)⁷.

Of all human IgG molecules, IgG3 shows the highest Fab-Fab folding flexibility and Fab Fc folding flexibility. The architecture of IgG3 is unique, as the hinge of IgG3, in contrast to all other immunoglobulins, incorporates 3 copies of a 15 aa motif caused by exon multiplication⁸⁻¹¹. Naturally occurring variants of IgG3 bearing only one or two copies of this motif in their hinge region show a much smaller distance between Fab and Fc (45 Å and 65 Å compared to 105 Å)⁸. These graduated differences and the opportunity of prolonging and shortening a spacer region by addition or removal of one or more copies of this 15 aa motif led in the present invention to the construction of an IgG3 Hinge library, using that, the length of the spacer can be fine-tuned to an optimal setting for every target. In addition, the inventors identified a monoclonal antibody that is specific to the IgG3 middle hinge motifs, allowing exploitation for additional, antigen-independent though CAR-specific functions, including purification, stimulation, expansion and depletion of CAR T cells.

SUMMARY

In one aspect, the present disclosure refers to an immunoreceptor, comprising one or more IgG3 middle hinge repeat domain motifs, wherein the immunoreceptor does not comprise an IgG3 CH2 and/or CH3 domain, wherein the immunoreceptor comprises an amino acid sequence which has at least 80% sequence identity, preferably at least 90% sequence identity, or most preferably 100% sequence identity with the amino acid sequence of [A-Bn], Wherein A is the amino acid sequence of SEQ ID NO: 2; B is said IgG3 middle hinge domain repeat motif, wherein said motif has the amino acid sequence of SEQ ID NO: 1; and n is

selected from the group consisting of 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 and is preferably an integer between 1 and 15 excluding 3, more preferably an integer between 1 and 10 excluding 3, even more preferably an integer between 1 and 5 excluding 3, most preferably 4 or 5.

In one aspect, the present disclosure refers to a CAR disclosed herein, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, and 171.

In one aspect, the present disclosure refers to a nucleic acid encoding the immunoreceptor or CAR disclosed herein.

In one aspect, the present disclosure refers to a cell, comprising the nucleic acid disclosed herein, optionally wherein: I) The cell is an immune cell, preferably a B cell, macrophage, NK cell or T cell, more preferably T cell, and even more preferably a CD4+ and/or CD8+ T cell; II) The cell expresses the immunoreceptor or CAR disclosed herein; III) The cell comprises the nucleic acid stably integrated into the genome; and/or IV) The nucleic acid comprised in the cell is comprised in an episomal vector.

In one aspect, the present disclosure refers to use of the nucleic acid, cell comprising the nucleic acid, immunoreceptor, or CAR, disclosed herein in the manufacture of a medicament for treating a cancer, an autoimmune disease, an infectious disease or a degenerative disease, optionally wherein the disease is a cancer, wherein the cancer is a hematological cancer or a solid cancer, optionally wherein the hematological cancer is leukemia or lymphoma, preferably acute myeloid leukemia, multiple myeloma, non-Hodgkin-lymphoma, Burkitt's lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, or diffuse large B cell lymphoma; optionally wherein the solid cancer is breast cancer, colon carcinoma, lung cancer, pancreatic or prostate cancer or glioblastoma.

In one aspect, the present disclosure refers to an antigen-binding protein, streptamer or aptamer which is capable of binding to an epitope comprised by a sequence consisting of at least one, preferably at least two, more preferably at least three repeats of the amino acid sequence of SEQ NO: 1, optionally wherein at least two repeats are adjacent to each other,

optionally wherein the antigen-binding protein, streptamer or aptamer is capable of binding to the immunoreceptor or CAR disclosed herein, optionally wherein the antigen-binding protein, streptamer or aptamer is capable of stimulating the immunoreceptor or CAR disclosed herein; and/or wherein the antigen-binding protein, streptamer or aptamer is an antigen-binding protein which is an antibody or fragment thereof, preferably a monoclonal antibody or fragment thereof; and/or wherein the antigen-binding protein comprises a) a heavy chain variable region having at least 80%, preferably at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 19, and wherein the heavy chain variable region preferably contains a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; and b) a light chain variable region having at least 80%, preferably at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 23, wherein the light chain variable region preferably contains a CDR1 having the amino acid sequence of SEQ ID NO: 24, a CDR2 having the amino acid sequence of SEQ ID NO: 25, and a CDR3 having the amino acid sequence of SEQ ID NO: 26.

In one aspect, the present disclosure refers to use of the antigen-binding protein, streptamer or aptamer disclosed herein for purification, detection, depletion, stimulation, expansion, or enrichment of cells expressing the immunoreceptor or CAR disclosed herein, wherein the use is *in vitro*.

In one aspect, the present disclosure refers to an *in vitro* method, comprising the step of: Binding an antigen-binding protein, streptamer or aptamer to cells expressing the immunoreceptor or CAR disclosed herein, preferably wherein the binding is binding specifically to the IgG3 middle hinge repeat domain comprised in said immunoreceptor or CAR, and/or wherein the antigen-binding protein, streptamer or aptamer is an antigen-binding protein, streptamer or aptamer disclosed herein, optionally wherein: 1) the method is a method of purification of cells expressing the immunoreceptor or CAR disclosed herein, comprising the steps of: A) Optionally obtaining the cells expressing the chimeric antigen receptor; B) Incubating said cells with a primary antibody, streptamer or aptamer, wherein the primary antibody, streptamer or aptamer is said antigen-binding protein, streptamer or aptamer disclosed herein, under conditions which allow the antibody, streptamer or aptamer to bind to the immunoreceptor or CAR expressed by the cells; C) Separating the antibody-, streptamer- or aptamer-bound cells from the non- bound cells in order to obtain the purified cells, optionally wherein step C comprises incubating the cells of step B with an entity capable of binding to the antibody, streptamer or aptamer; and wherein I) The entity is preferably a secondary antibody, more preferably labelled with a fluorescent marker; or a bead, more preferably a magnetic bead; II) The primary antibody, streptamer or aptamer is labelled, wherein the label is preferably a tag or a fluorescent dye; III) The separation of step C is carried out by means of MACS or FACS; and/or IV) Wherein the separation is carried out using a Streptamer or an Aptamer; or 2) the method is a method of depletion of cells

expressing the immunoreceptor or CAR disclosed herein, comprising the steps of: A) Optionally obtaining the cells expressing the immunoreceptor or CAR; and B) Incubating said cells with an antigen-binding protein, streptamer or aptamer disclosed herein coupled to a cytotoxic molecule; or 3) the method is a method of a) stimulation and/or b) expansion of cells expressing the immunoreceptor or CAR disclosed herein, comprising the steps of: A) Optionally obtaining the cells expressing the immunoreceptor or CAR; and B) Incubating said cells with an antigen-binding protein, streptamer or aptamer disclosed herein, optionally wherein the antigen-binding protein, streptamer or aptamer is coupled to a solid phase, or wherein the antigen-binding protein, streptamer or aptamer is expressed on the surface of a cell, optionally wherein: I) The solid phase is a tissue culture surface or a bead, preferably a magnetic bead; and/or II) The solid phase is a scaffold consisting of polymers, preferably starch or sugar; or 4) the method is a method of enrichment of cells expressing the immunoreceptor or CAR disclosed herein, comprising the steps of: A) Stimulating and/or expanding the cells according to the method disclosed herein; and B) Purifying the cells of step A according to the method disclosed herein.

In one aspect, the present disclosure refers to a pharmaceutical composition, comprising the antigen-binding protein, streptamer or aptamer disclosed herein or a cell expressing a chimeric antigen receptor comprising all or part of said antigen-binding protein, streptamer or aptamer, the composition optionally further comprising a pharmaceutically acceptable carrier and/or excipient.

In one aspect, the present disclosure refers to use of the antigen-binding protein, streptamer or aptamer disclosed herein or a cell expressing a chimeric antigen receptor comprising all or part of said antigen-binding protein, streptamer or aptamer, or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for depletion of cells expressing the immunoreceptor or CAR disclosed herein, wherein said antigen-binding protein, streptamer or aptamer coupled to a cytotoxic molecule or cells expressing said chimeric antigen receptor comprising said all or part of said antigen-binding protein, streptamer or aptamer is to be administered to a subject in need thereof.

In one aspect, the present disclosure refers to a kit, comprising the immunoreceptor or CAR disclosed herein and the antigen-binding protein, streptamer or aptamer disclosed herein.

In one aspect, the present disclosure refers to a bispecific antibody, comprising one or more IgG3 middle hinge repeat domain motifs, optionally wherein the one or more IgG3 middle hinge domain repeat motifs I) Are from a human IgG3 middle hinge; and/or II) Consist of the amino acid sequence of SEQ ID NO: 1; and/or III) Have reduced immunogenicity compared to repeats of an IgG1 hinge domain and/or an IgG4 hinge domain; and/or wherein the bispecific antibody: I) Does not comprise all or part of the sequence of the lower hinge domain of a human IgG3 hinge domain; II) Comprises an amino acid sequence which has at least 80% sequence identity, preferably at least 90% sequence identity, or most preferably 100% sequence identity with the amino acid sequence of [A-Bn], wherein A is the amino acid sequence of SEQ ID NO: 2; B is said IgG3 middle hinge domain repeat motif, wherein said motif has the amino acid sequence of SEQ ID NO: 1; and n is selected from the group consisting of 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 and is preferably an integer between 1 and 15 excluding 3, more preferably an integer between 1 and 10 excluding 3, even more preferably an integer between 1 and 5 excluding 3, most preferably 4 or 5; III) Comprises the IgG3 middle hinge domain repeat motif 1, 2, 4, 5, 6, 7, 8, 9 or 10 times; and/or IV) has reduced immunogenicity compared to a second bispecific antibody which differs from the first bispecific antibody in that it does not comprise said one or more IgG3 middle hinge domain repeat; and/or wherein the bispecific antibody comprises at least two, preferably at least three IgG3 middle hinge repeat domain motifs, optionally wherein at least two of said IgG3 middle hinge repeat domain motifs are adjacent to each other.

DESCRIPTION OF THE INVENTION

The invention generally relates to immunotherapy using immune cells such as chimeric antigen receptor (CAR)-engineered T cells. In particular, the invention relates to immunotherapy using chimeric antigen receptor (CAR)-engineered T cells that carry a novel, IgG3-Hinge-based spacer domain, allowing a finely modulated response to target antigens. In addition, the invention relates to the introduction of one or more IgG3-Hinge-based multi-function sites (MFS) into CARs and other immunoreceptors, allowing purification, stimulation, expansion and depletion of CAR T cells. The invention includes also the

CLAIMS

1. An immunoreceptor, comprising one or more IgG3 middle hinge repeat domain motifs, wherein the immunoreceptor does not comprise an IgG3 CH2 and/or CH3 domain.

~~2. The immunoreceptor according to claim 1~~, wherein the immunoreceptor comprises an amino acid sequence which has at least 80% sequence identity, preferably at least 90% sequence identity, or most preferably 100% sequence identity with the amino acid sequence of [A-B_n],

wherein

A is the amino acid sequence of SEQ ID NO: 2;

B is said IgG3 middle hinge domain repeat motif, wherein said motif has the amino acid sequence of SEQ ID NO: 1; and

n is selected from the group consisting of ~~0~~, 1, 2, ~~3~~, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 and is preferably an integer between 1 and 15 excluding 3, more preferably an integer between 1 and 10 excluding 3, even more preferably an integer between 1 and 5 excluding 3, most preferably ~~an integer between 3 and 4 or~~ 5.

~~3. The immunoreceptor according to claim 2, wherein the immunoreceptor comprises an amino acid sequence which has 100% sequence identity with the amino acid sequence of [A-B_n].~~

~~4. The immunoreceptor according to claim 2 or 3, wherein n is an integer between 1 and 10.~~

~~5. The immunoreceptor according to claim 2 or 3, wherein n is an integer between 1 and 5.~~

~~6. The immunoreceptor according to claim 2 or 3, wherein n is an integer between 3 and 5.~~

27. The immunoreceptor according to any one of the preceding claims, comprising:

an extracellular antigen-binding domain,

a spacer domain,

a transmembrane domain, and

an intracellular signaling domain;

wherein the spacer domain is located between the extracellular antigen-binding domain and the transmembrane domain,

and wherein optionally the spacer domain comprises one or more IgG3 middle hinge domain repeat motifs-

~~8. The immunoreceptor according to claim 7,~~ optionally wherein the transmembrane domain and the intracellular domain together consist of a sequence selected from the group consisting of SEQ ID NO: 109, 110, 111, 112, 113, 114, 115 and 174.

39. The immunoreceptor according to any one of the preceding claims, comprising an extracellular antigen-binding domain comprising:

a first domain,

a linker, and, optionally,

a second domain;

optionally wherein the linker is located between the first domain and the second domain,

and wherein optionally the linker comprises one or more IgG3 middle hinge domain repeat motifs-

~~10. The immunoreceptor according to claims 7, 8 or 9,~~
; and/or

wherein the spacer domain comprises one or more IgG3 middle hinge domain repeat motifs,

and/or

wherein the linker comprised in the extracellular antigen-binding domain comprises one or more IgG3 middle hinge domain repeat motifs.

411. The immunoreceptor according to any one of the preceding claims, wherein the immunoreceptor is selected from the group consisting of a T-cell receptor (TCR), preferably a recombinant TCR; a B-cell receptor (BCR), preferably a recombinant BCR; and a chimeric antigen receptor (CAR).

512. The immunoreceptor according to any one of claims ~~9 to 113~~ or 4, wherein the immunoreceptor comprises the antigen-binding domain, wherein

- I) the first domain comprises a heavy chain variable domain;
- II) the first domain comprises a light chain variable domain;
- III) the first domain comprises a heavy chain variable domain, and the second domain comprises a light chain variable domain;
- IV) the first domain comprises a heavy chain variable domain, and the second domain comprises a heavy chain variable domain; or
- V) the first domain comprises a light chain variable domain, and the second domain comprises a light chain variable domain.

613. The immunoreceptor according to any one of claims ~~9-3~~ to ~~125~~, wherein the immunoreceptor comprises the antigen-binding domain, said antigen-binding domain comprising the first domain, linker, and second domain, which are part of a single chain variable fragment (scFv),
wherein the scFv optionally comprises, as heavy/light chain variable sequences comprised in the first/second domain, heavy/light chain variable sequences of scFvs specific for one of the following antigens:

- A) CD19, optionally wherein
 - i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 27,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 28,
and the scFv is capable of specifically binding to CD19; or
 - ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 27 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 28;
- B) CD20, optionally wherein

- i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 30,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 29,
and the scFv is capable of specifically binding to CD20; or
 - ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 30 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 29;
- C) ROR1, optionally wherein
 - i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 31, 33, 35, or 37,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 32, 34, 36, or 38, respectively,
and the scFv is capable of specifically binding to ROR1; or
 - ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 31, 33, 35, or 37 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 32, 34, 36, 38, respectively;
- D) ROR2, optionally wherein
 - i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 39,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 40,
and the scFv is capable of specifically binding to ROR2; or

- ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 39 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 40;
- E) SLAMF7, optionally wherein
 - i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 41 or 43,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 42 or 44, respectively,
and the scFv is capable of specifically binding to SLAMF7; or
 - ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 41 or 43 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 42 or 44, respectively;
- F) FLT3, optionally wherein
 - i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 45 or 47,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 46 or 48, respectively,
and the scFv is capable of specifically binding to FLT3; or
 - ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 45 or 47 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 46 or 48, respectively;
- G) Siglec-6, optionally wherein
 - i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 49,

- the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 50,
and the scFv is capable of specifically binding to Siglec-6; or
- ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 49 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 50;
- H) $\alpha_v\beta_3$ integrin, optionally wherein
- i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 51 or 53,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 52 or 54, respectively,
and the scFv is capable of specifically binding to $\alpha_v\beta_3$ integrin; or
- ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 51 or 53 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 52 or 54, respectively;
- or
- I) BCMA, optionally wherein
- i) the heavy chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 55 or 57,
the light chain variable domain has an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 56 or 58, respectively,
and the scFv is capable of specifically binding to BCMA; or
- ii) the heavy chain variable domain has the amino acid sequence of SEQ ID NO: 55 or 57 and the light chain variable domain has the amino acid sequence of SEQ ID NO: 56 or 58, respectively;

~~14. The immunoreceptor according to any one of claims 9 to 13, and/or~~

wherein the immunoreceptor comprises the antigen-binding domain, said antigen-binding domain comprising an scFv:

- I.) specific to CD19, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 3 or 71 and is capable of specifically binding to CD19, or wherein said scFv has the amino acid sequence of SEQ ID NO: 3 or 71;
- II.) specific to CD20 optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 4 or 72 and is capable of specifically binding to CD20, or wherein said scFv has the amino acid sequence of SEQ ID NO: 4 or 72;
- III.) specific to ROR1, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 5, 6, 7, 8, 73, 74, 75, 76, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100 and is capable of specifically binding to ROR1, or wherein said scFv has the amino acid sequence of SEQ ID NO: 5, 6, 7, 8, 73, 74, 75, 76, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100;
- IV.) specific to ROR2, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 9, 77, 101, 102, 103, 104, 105, 106, 107 or 108 and is capable of specifically binding to ROR2, or wherein said scFv has the amino acid sequence of SEQ ID NO: 9, 77, 101, 102, 103, 104, 105, 106, 107 or 108;
- V.) specific to SLAMF7, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 10, 11, 78 or 79 and is capable of specifically binding to SLAMF7, or wherein said scFv has the amino acid sequence of SEQ ID NO: 10, 11, 78 or 79;

- VI.) specific to FLT3, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 12, 13, 80 or 81 and is capable of specifically binding to FLT3, or wherein said scFv has the amino acid sequence of SEQ ID NO: 12, 13, 80 or 81;
- VII.) specific to Siglec-6, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 14 or 82 and is capable of specifically binding to Siglec-6, or wherein said scFv has the amino acid sequence of SEQ ID NO: 14 or 82;
- VIII.) specific to $\alpha_v\beta_3$ integrin, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 15, 16, 83 or 84 and is capable of specifically binding to $\alpha_v\beta_3$ integrin, or wherein said scFv has the amino acid sequence of SEQ ID NO: 15, 16, 83 or 84;
- IX.) specific to BCMA, optionally wherein said scFv comprises an amino acid sequence having at least 80% sequence identity, preferably at least 90% sequence identity, to SEQ ID NO: 17, 18, 85 or 86 and is capable of specifically binding to BCMA, or wherein said scFv has the amino acid sequence of SEQ ID NO: 17, 18, 85 or 86.

~~715.~~ The immunoreceptor according to any one claims 1 to ~~614~~, wherein the immunoreceptor is a chimeric antigen receptor (CAR);

and/or

~~16. The immunoreceptor or CAR according to any one of claims 1 to 15,~~ wherein the one or more IgG3 middle hinge domain repeat motifs

- I) Are from a human IgG3 middle hinge; and/or
- II) Consist of the amino acid sequence of SEQ ID NO: 1; and/or
- III) Have reduced immunogenicity compared to repeats of an IgG1 hinge domain and/or an IgG4 hinge domain;

and/or

~~17. The immunoreceptor or CAR according to any one of claims 1 to 16,~~ wherein the immunoreceptor or CAR:

- I) Does not comprise all or part of the sequence of the lower hinge domain of a human IgG3 hinge domain;
- II) Comprises an amino acid sequence which has at least 80% sequence identity, preferably at least 90% sequence identity, or most preferably 100% sequence identity with the amino acid sequence of [A-B_n], wherein
A is the amino acid sequence of SEQ ID NO: 2;
B is said IgG3 middle hinge domain repeat motif, wherein said motif has the amino acid sequence of SEQ ID NO: 1; and
n is selected from the group consisting of ~~0~~, 1, 2, ~~3~~, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 and is preferably an integer between 1 and 15 excluding 3, more preferably an integer between 1 and 10 excluding 3, even more preferably an integer between 1 and 5 excluding 3, most preferably ~~an integer between 3 and 5~~ and/or 5;
- III) Comprises the IgG3 middle hinge domain repeat motif 1, 2, ~~3~~, 4, 5, 6, 7, 8, 9 or 10 times; and/or
- IV) has reduced immunogenicity compared to a second CAR which differs from the first CAR in that it does not comprise said one or more IgG3 middle hinge domain repeat motifs;

and/or

~~18. The immunoreceptor or CAR according to any one of claims 1 to 17,~~ wherein the immunoreceptor or CAR comprises at least two, preferably at least three of said IgG3 middle hinge domain repeat motifs which are adjacent to each other.

819. A CAR according to any one of the preceding claims, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154,

155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, and 171.

920. A nucleic acid encoding the immunoreceptor or CAR according to any one of claims 1 to 819.

1021. A cell, comprising the nucleic acid according to claim 920.

~~22. The cell according to claim 21, optionally~~ wherein:

- I) The cell is an immune cell, preferably a B cell, macrophage, NK cell or T cell, more preferably T cell, and even more preferably a CD4⁺ and/or CD8⁺ T cell;
- II) The cell expresses the immunoreceptor or CAR according to any one of claims 1 to 819;
- III) The cell comprises the nucleic acid stably integrated into the genome; and/or
- IV) The nucleic acid comprised in the cell is comprised in an episomal vector.

1123. ~~Use of t~~The nucleic acid, cell comprising the nucleic acid, immunoreceptor, or CAR, according to any one of claims 1 to 1022 for use in a method of in the manufacture of a medicament for treating a cancer, an autoimmune disease, an infectious disease or a degenerative disease.

~~24. The immunoreceptor, CAR, nucleic acid or cell comprising the nucleic acid for use of claim 23, optionally~~ wherein the disease is a cancer, wherein the cancer is ~~is~~ a hematological cancer or a solid cancer, optionally wherein the hematological cancer is leukemia or lymphoma, preferably acute myeloid leukemia, multiple myeloma, non-Hodgkin-lymphoma, Burkitt's lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, or diffuse large B cell lymphoma; optionally wherein the solid cancer is breast cancer, colon carcinoma, lung cancer, pancreatic or prostate cancer or glioblastoma.

1225. An antigen-binding protein, streptamer or aptamer which is capable of binding to an epitope comprised by a sequence consisting of at least one, preferably at least two, more preferably at least three repeats of the amino acid sequence of SEQ NO: 1, optionally wherein at least two repeats are adjacent to each other.

~~26. The antigen-binding protein, streptamer or aptamer of claim 25, optionally~~ wherein the antigen-binding protein, streptamer or aptamer is capable of binding to the immunoreceptor or CAR according to any one of claims 1 to ~~819~~.

~~27. The antigen-binding protein, streptamer or aptamer of claim 26, optionally~~ wherein the antigen-binding protein, streptamer or aptamer is capable of stimulating the immunoreceptor or CAR according to any one of claims 1 to ~~819~~;

~~and/or~~

~~28. The antigen-binding protein, streptamer or aptamer according to any one of claims 25 to 27,~~ wherein the antigen-binding protein, streptamer or aptamer is an antigen-binding protein which is an antibody or fragment thereof, preferably a monoclonal antibody or fragment thereof;

~~and/or~~

~~29. The antigen-binding protein of any one of claims 25 to 28,~~ wherein the antigen-binding protein comprises

- a) a heavy chain variable region having at least 80%, preferably at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 19, and wherein the heavy chain variable region preferably contains a CDR1 having the amino acid sequence of SEQ ID NO: 20, a CDR2 having the amino acid sequence of SEQ ID NO: 21, and a CDR3 having the amino acid sequence of SEQ ID NO: 22; and
- b) a light chain variable region having at least 80%, preferably at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 23, wherein the light chain variable region preferably contains a CDR1 having the amino acid sequence of SEQ ID NO: 24, a CDR2 having the amino acid sequence of SEQ ID NO: 25, and a CDR3 having the amino acid sequence of SEQ ID NO: 26.

1330. Use of the antigen-binding protein, streptamer or aptamer according to ~~any one of claims 25 to 29~~12 for purification, detection, depletion, stimulation, expansion, or enrichment of cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to ~~819~~, wherein the use is in vitro.

1431. An in vitro method, comprising the step of:

Binding an antigen-binding protein, streptamer or aptamer to cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to ~~819~~, preferably wherein the binding is binding specifically to the IgG3 middle hinge repeat domain comprised in said immunoreceptor or CAR, and/or wherein the antigen-binding protein, streptamer or aptamer is an antigen-binding protein, streptamer or aptamer as defined in ~~any one of claims 25 to 2912~~,

optionally wherein:

~~32. The method of claim 31, wherein~~1) the method is a method of purification of cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to ~~819~~, comprising the steps of:

- A) Optionally obtaining the cells expressing the chimeric antigen receptor;
- B) Incubating said cells with a primary antibody, streptamer or aptamer, wherein the primary antibody, streptamer or aptamer is said antigen-binding protein, streptamer or aptamer as defined in ~~any one of claims 25 to 2912~~, under conditions which allow the antibody, streptamer or aptamer to bind to the immunoreceptor or CAR expressed by the cells;
- C) Separating the antibody-, streptamer- or aptamer-bound cells from the non-bound cells in order to obtain the purified cells.

~~33. The purification method of claim 32,~~optionally wherein step C comprises incubating the cells of step B with an entity capable of binding to the antibody, streptamer or aptamer; and wherein

- I) The entity is preferably a secondary antibody, more preferably labelled with a fluorescent marker; or a bead, more preferably a magnetic bead;
 - II) The primary antibody, streptamer or aptamer is labelled, wherein the label is preferably a tag or a fluorescent dye;
 - III) The separation of step C is carried out by means of MACS or FACS; and/or
 - IV) Wherein the separation is carried out using a Streptamer or an Aptamer;
- or

~~34. The method of claim 31, wherein²⁾~~ the method is a method of depletion of cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to ~~819~~, comprising the steps of:

- A) Optionally obtaining the cells expressing the immunoreceptor or CAR; and
- B) Incubating said cells with an antigen-binding protein, streptamer or aptamer as defined in ~~claim 12 any one of claims 25 to 29~~ coupled to a cytotoxic molecule;_{:-}

or

~~35. The method of claim 31, wherein³⁾~~ the method is a method of a) stimulation and/or b) expansion of cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to ~~819~~, comprising the steps of:

- A) Optionally obtaining the cells expressing the immunoreceptor or CAR; and
- B) Incubating said cells with an antigen-binding protein, streptamer or aptamer as defined in ~~any one of claims 25 to 29~~¹², optionally wherein the antigen-binding protein, streptamer or aptamer is coupled to a solid phase, or wherein the antigen-binding protein, streptamer or aptamer is expressed on the surface of a cell.

~~36. The stimulation or expansion method of claim 35, optionally~~ wherein:

- I) The solid phase is a tissue culture surface or a bead, preferably a magnetic bead; and/or
- II) The solid phase is a scaffold consisting of polymers, preferably starch or sugar;_{:-}

or

~~37. The method of claim 31, wherein⁴⁾~~ the method is a method of enrichment of cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to ~~819~~, comprising the steps of:

- A) Stimulating and/or expanding the cells according to the method defined in the above claim item 3~~of claims 35 or 36~~; and
- B) Purifying the cells of step A according to the method defined in the above claim item 1~~of claim 32 or 33~~.
- ~~38. The method or use of any one of claims 30 to 37, wherein said method or use is an *in vitro* method or use.~~
- ~~39. The method or use of any one of claims 30 to 38, wherein said method or use does not comprise a method for treatment of the human or animal body by surgery or therapy or a diagnostic method practised on the human or animal body.~~
1540. A pharmaceutical composition, comprising the antigen-binding protein, streptamer or aptamer according to ~~any one of claims 25 to 29~~12 or a cell expressing a chimeric antigen receptor comprising all or part of said antigen-binding protein, streptamer or aptamer, the composition optionally further comprising a pharmaceutically acceptable carrier and/or excipient.
1641. ~~Use of t~~The antigen-binding protein, streptamer or aptamer according to ~~any one of claims 25 to 29~~12 or a cell expressing a chimeric antigen receptor comprising all or part of said antigen-binding protein, streptamer or aptamer, or the pharmaceutical composition of claim 1640, in the manufacture of a medicament for ~~use in a therapeutic method of~~ depletion of cells expressing the immunoreceptor or CAR as defined in any one of claims 1 to 819, ~~comprising administering to a subject in need thereof~~wherein said antigen-binding protein, streptamer or aptamer coupled to a cytotoxic molecule or cells expressing said chimeric antigen receptor comprising said all or part of said antigen-binding protein, streptamer or aptamer is to be administered to a subject in need thereof.
1742. A kit, comprising the immunoreceptor or CAR as defined in any one of claims 1 to 819 and the antigen-binding protein, streptamer or aptamer as defined in ~~any one of claims 25 to 27~~12.
1843. A bispecific antibody, comprising one or more IgG3 middle hinge repeat domain motifs.
- ~~44. The bispecific antibody according to claim 43, optionally~~ wherein the one or more IgG3 middle hinge domain repeat motifs

- I) Are from a human IgG3 middle hinge; and/or
- II) Consist of the amino acid sequence of SEQ ID NO: 1; and/or
- III) Have reduced immunogenicity compared to repeats of an IgG1 hinge domain and/or an IgG4 hinge domain;

and/or

~~45. The bispecific antibody according to claims 43 or 44, wherein the bispecific antibody:~~

- I) Does not comprise all or part of the sequence of the lower hinge domain of a human IgG3 hinge domain;
- II) Comprises an amino acid sequence which has at least 80% sequence identity, preferably at least 90% sequence identity, or most preferably 100% sequence identity with the amino acid sequence of [A-B_n], wherein
A is the amino acid sequence of SEQ ID NO: 2;
B is said IgG3 middle hinge domain repeat motif, wherein said motif has the amino acid sequence of SEQ ID NO: 1; and
n is selected from the group consisting of ~~0, 1~~, 2, ~~3~~, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 and is preferably an integer between 1 and 15 excluding 3, more preferably an integer between 1 and 10 excluding 3, even more preferably an integer between 1 and 5 excluding 3, most preferably ~~4 an integer between 3 and or~~ 5;
- III) Comprises the IgG3 middle hinge domain repeat motif 1, 2, ~~3~~, 4, 5, 6, 7, 8, 9 or 10 times; and/or
- IV) has reduced immunogenicity compared to a second bispecific antibody which differs from the first bispecific antibody in that it does not comprise said one or more IgG3 middle hinge domain repeat ~~motifs;~~

~~46. The bispecific antibody according to any one of claims 43 to 45, wherein the immunoreceptor comprises an amino acid sequence which has 100% sequence identity with the amino acid sequence of [A-B_n].~~

- ~~47. The bispecific antibody according to claim 45 or 46, wherein n is an integer between 1 and 10.~~
- ~~48. The immunoreceptor according to claim 45 or 46, wherein n is an integer between 1 and 5.~~
- ~~49. The immunoreceptor according to claim 45 or 46, wherein n is an integer between 3 and 5.~~
- ~~50. The bispecific antibody according to any one of claims 43 to 49, and/or wherein the bispecific antibody comprisesing at least two, preferably at least three IgG3 middle hinge repeat domain motifs, optionally wherein at least two of said IgG3 middle hinge repeat domain motifs are adjacent to each other.~~
1951. The immunoreceptor, CAR, nucleic acid, cell, method, pharmaceutical composition, kit or bispecific antibody according to any one of claims 1 to 1124 or 1431 to 1950, wherein the IgG3 middle hinge repeat domain motif is not a mouse IgG3 middle hinge repeat domain.