

to form an artificial immune synapse eventually leading to the destruction of the cell expressing said target antigen.

[3] From a clinical perspective, TCEs do not only have to show efficient cell killing activity, but at the same time other characteristics like, e.g., a manageable side effect profile are desired. However, these aspects are not the only considerations when it comes to the development of TCEs. Other areas of importance for drug development are pre-patient, i.e. concern characteristics of the TCE outside of the patient's body such as, e.g., shelf life, production scalability, solubility, stability, and ease of formulation. In other terms, an important feature from a pharmaceutical perspective is the developability of a given TCE or parts thereof to address certain needs such as for example in drug production or administration. Bringing together the requirements from clinical and pharmacological perspective is important for TCE platform advancement in relation to commercialization. In view of the biological complexities of having the TCE inducing the formation of the immune synapse, it can be challenging to manipulate the TCE in terms of its other characteristics such as for example shelf life, production scalability, solubility, stability, and ease of formulation while maintaining its effectiveness in mediating the killing of target cells.

[4] Thermal stability of TCEs is an important feature within and outside of the patient's body. A higher thermal stability of TCEs reduces the formation of high molecular weight aggregates caused which in turn provides, e. g., for a TCE that is active for a prolonged time in the body but will also be more stable during storage. Moreover, the reduction of high molecular weight aggregates can also result in a more favourable immunogenetic profile of the TCE upon administration thereby reducing the risk of side effects.

[5] Classically, the VH and VL domains making up the anti-target and anti-CD3 binding domains of TCEs are an area that has been the focus of improving stability. This is in particular true for Fvs that are part of TCEs. The hydrophobic interaction between the VH and the VL is believed to strongly determine the stability of a Fv. The interaction can in many Fvs not be strong enough to be stable for in vitro and/or in vivo applications. This has led to the coupling of the V-domains of an Fv by a linker resulting in single chain Fvs (scFvs). While the linker has shown to be effective in holding the V-domains (variable domains) together, it may not always be effective to keep a scFv active at all or active enough for a given application making it necessary to further improve stability. One approach to increase stability is that by increasing the interaction of the two V-domains, the stability of a biologically active Fv can be increased so that it remains a target binding monomer. Although approaches have been described in the art to improve stability of scFv, there is still a need in the art for improving stability of Fvs that are part of TCEs.

Summary

[5a] In one aspect, the present disclosure refers to a polypeptide or polypeptide construct comprising:

- 5 a binding domain binding to an extracellular epitope of the human CD3ε chain comprising or consisting of a VH region and a VL region, wherein
- i) the VH region comprises:
- a CDR-H1 sequence of X₁YAX₂N, where X₁ is K, V, S, G, R, T, or I; and X₂ is M or I;
- 10 a CDR-H2 sequence of RIRSKYNNYATYYADX₁VKX₂, where X₁ is S or Q; and X₂ is D, G, K, S, or E; and
- a CDR-H3 sequence of HX₁NFGNSYX₂SX₃X₄AY, where X₁ is G, R, or A; X₂ is I, L, V, or T; X₃ is Y, W or F; and X₄ is W, F or Y; and
- ii) wherein the VL region comprises:
- 15 a CDR-L1 sequence of X₁SSTGAVTX₂X₃X₄YX₅N, where X₁ is G, R, or A; X₂ is S or T; X₃ is G or S; X₄ is N or Y; and X₅ is P or A;
- a CDR-L2 sequence of X₁TX₂X₃X₄X₅X₆, where X₁ is G or A; X₂ is K, D, or N; X₃ is F, M or K; X₄ is L or R; X₅ is A, P, or V; and X₆ is P or S; and
- a CDR-L3 sequence of X₁LWYSNX₂WV, where X₁ is V, A, or T; and X₂ is R or L; and
- 20 iii) wherein one or more of CDR sequences of the VH region of i) and/or of the VL region of ii) comprise one amino acid substitution or a combination thereof selected from X₂4V and X₂4F in CDR-H1;
- D15, and X₁16A in CDR-H2;
- 25 H1, X₁2E, F4, and N6 in CDR-H3; and
- X₁1L and W3 in CDR-L3.

[5b] In another aspect, the present disclosure refers to a polypeptide or polypeptide construct comprising: a binding domain binding to an extracellular epitope of the human CD3ε chain comprising or consisting of a VH region and a VL region, wherein

- 30 i) the VH region comprises the sequence of
- EVX₁LX₂ESGGGLX₃QPX₄GSLKLSAASGFTFNX₅YAX₆NWVRQAPGKGLEWVAR
- IRSKYNNYATYYADX₇VKX₈RFTISRDDSX₉X₁₀X₁₁X₁₂YLQMNNLKTEDTAX₁₃YYCV
- RHX₁₄NFGNSYX₁₅SX₁₆X₁₇AYWGQGTTLTVSX₁₈, where X₁ is Q or K; X₂ is V or L;
- 35 X₃ is V or E; X₄ is G or K; X₅ is K, V, S, G, R, T, or I; X₆ is M or I; X₇ is S or Q; X₈ is D, G, K, S, or E; X₉ is K or Q; X₁₀ is N or S; X₁₁ is T or I; X₁₂ is A or L; X₁₃ is V or M;
- X₁₄ is G, R or A; X₁₅ is I, L, V, or T; X₁₆ is Y, W or F; X₁₇ is W, F or Y; and X₁₈ is S or A; and
- ii) the VL region comprises the sequence of
- 40 QX₁VVTQEX₂X₃LTX₄SPGX₅TVTLTCX₆SSTGAVTX₇X₈X₉YX₁₀NWVQX₁₁KPX₁₂X₁₃X₁₄X₁₅X₁₆GLIGX₁₇TX₁₈X₁₉X₂₀X₂₁X₂₂GX₂₃PARFSGSLX₂₄GX₂₅KAALTX₂₆X₂₇GX₂₈QX₂₉E
- DEAX₃₀YX₃₁CX₃₂LWYSNX₃₃WVFGGGTKLTVL, where X₁ is T or A; X₂ is P or S; X₃ is S or A; X₄ is V or T; X₅ is G or E; X₆ is G, R, or A; X₇ is S or T; X₈ is G or S; X₉ is N or Y; X₁₀ is P or A; X₁₁ is Q or E; X₁₂ is G or D; X₁₃ is Q or H; X₁₄ is A or L; X₁₅ is P or F; X₁₆ is R or T; X₁₇ is G or A; X₁₈ is K or D; X₁₉ is F or M; X₂₀ is L or R; X₂₁ is A, P or V; X₂₂ is P or S; X₂₃ is T or V; X₂₄ is L or I; X₂₅ is G or D; X₂₆ is L or I; X₂₇ is S or T; X₂₈ is V or A; X₂₉ is P or T; X₃₀ is E or I; X₃₁ is Y or F; X₃₂ is V, A, or T; X₃₃ is R or L; and
- 45

iii) wherein the VH and/or VL region sequence comprises one amino acid substitution or a combination thereof selected from

a. N30, X₆34V, X₆34F, Q39, L45, D64, X₇65A, X₁₂81V, X₁₂81T, X₁₂81L, V99, H101, X₁₄102E, F104, and N106 in the VH region sequence in i); and

b. L20, V38, X₁₁40R, X₁₁40K, X₂₄69, X₃₂91L, X₃₃93, and G102 in the VL region sequence in ii).

[5c] In another aspect, the present disclosure refers to a polynucleotide encoding a polypeptide or polypeptide construct as defined herein.

[5d] In another aspect, the present disclosure refers to a vector comprising a polynucleotide as defined herein.

[5e] In another aspect, the present disclosure refers to a host cell transformed or transfected with the polynucleotide as defined herein or with the vector as defined herein.

[5f] In another aspect, the present disclosure refers to a process for the production of an polypeptide or polypeptide construct as disclosed herein, said process comprising culturing a host cell as defined herein under conditions allowing the expression of the polypeptide or polypeptide construct as defined herein and recovering the produced polypeptide or polypeptide construct from the culture.

[5g] A pharmaceutical composition comprising a polypeptide or polypeptide construct according to any one of claims 1 to 20, or produced according to the process of claim 24.

[5h] The polypeptide or polypeptide construct as disclosed herein, or the polypeptide or polypeptide construct produced according to the process as disclosed herein, for use in therapy.

[5i] Use of the polypeptide or polypeptide construct as disclosed herein, or the polypeptide or polypeptide construct produced according to the process as disclosed herein in the manufacture of a medicament for the prevention, treatment or amelioration of a tumorous disease.

[5e] A kit comprising a polypeptide or polypeptide construct as disclosed herein, or produced according to the process as disclosed herein, a polynucleotide as defined herein, a vector as defined herein, and/or a host cell as defined herein.

Detailed Description

[6] The invention relates to a polypeptide or polypeptide construct comprising:

Claims

1. A polypeptide or polypeptide construct comprising:
 - 5 a binding domain binding to an extracellular epitope of the human CD3 ϵ chain comprising or consisting of a VH region and a VL region, wherein
 - i) the VH region comprises:
 - a CDR-H1 sequence of X₁YAX₂N, where X₁ is K, V, S, G, R, T, or I; and X₂ is M or I;
 - 10 a CDR-H2 sequence of RIRSKYNNYATYYADX₁VKX₂, where X₁ is S or Q; and X₂ is D, G, K, S, or E; and
 - a CDR-H3 sequence of HX₁NFGNSYX₂SX₃X₄AY, where X₁ is G, R, or A; X₂ is I, L, V, or T; X₃ is Y, W or F; and X₄ is W, F or Y; and
 - ii) wherein the VL region comprises:
 - 15 a CDR-L1 sequence of X₁SSTGAVTX₂X₃X₄YX₅N, where X₁ is G, R, or A; X₂ is S or T; X₃ is G or S; X₄ is N or Y; and X₅ is P or A;
 - a CDR-L2 sequence of X₁TX₂X₃X₄X₅X₆; where X₁ is G or A; X₂ is K, D, or N; X₃ is F, M or K; X₄ is L or R; X₅ is A, P, or V; and X₆ is P or S; and
 - a CDR-L3 sequence of X₁LWYSNX₂WV, where X₁ is V, A, or T; and X₂ is R or L;
 - 20 and
 - iii) wherein one or more of CDR sequences of the VH region of i) and/or of the VL region of ii) comprise one amino acid substitution or a combination thereof selected from X₂4V and X₂4F in CDR-H1;
 - D15, and X₁16A in CDR-H2;
 - 25 H1, X₁2E, F4, and N6 in CDR-H3; and
 - X₁1L and W3 in CDR-L3.
2. The polypeptide or polypeptide construct of claim 1, wherein in addition to said one amino acid substitution or a combination thereof as defined in iii) X₂ is I in said CDR-H1 sequence; X₂ is G in said CDR-H2 sequence; X₁ is A, X₄ is F in said CDR-H3 sequence; and/or X₁ is A in said CDR-L3 sequence.
3. The polypeptide or polypeptide construct of claim 1 or 2, comprising combinations of said amino acid substitutions as defined in iii) of two, three, four, five, six, seven, eight, nine, 35 ten, eleven, twelve or more amino acid substitutions.
4. The polypeptide or polypeptide construct of any one of claims 1 to 3, wherein:

- i) said one amino acid substitution is selected from:
 - a. X₂4V or X₂4F in CDR-H1; H1A in CDR-H3; and
 - b. X₁1L and W3Y in CDR-L3;
- ii) said combination of two or more amino acid substitutions are selected from X₁16A in CDR-H2 and N6S in CDR-H3; and X₁16A in CDR-H2 and N6T in CDR-H3;
- iii) said combination of three or more amino acid substitutions are selected from X₁16A in CDR-H2, H1A and N6S in CDR-H3; X₁16A in CDR-H2, H1A and N6T in CDR-H3; X₁16A in CDR-H2, H1N and N6T in CDR-H3;
- iv) said combination of four or more amino acid substitutions are selected from X₁16A in CDR-H2, H1A, N6S in CDR-H3, and W3Y in CDR-L3; D15E, X₁16A in CDR-H2, H1A, and N6T in CDR-H3; D15E, X₁16A in CDR-H2, H1A, and N6S in CDR-H3; X₁16A in CDR-H2, H1A, F4I and N6S in CDR-H3; or
- v) said combination of five or six amino acid substitutions are selected from X₁16A in CDR-H2, H1A, X₁2E, F4I, and N6T in CDR-H3; X₁16A in CDR-H2, H1N, X₁2E, F4I, and N6S in CDR-H3; D15E, X₁16A in CDR-H2, H1A, N6S in CDR-H3; and W3Y in CDR-L3; D15E, X₁16A in CDR-H2, H1N, X₁2E, F4I and N6T in CDR-H3; D15E, X₁16A in CDR-H2, H1A, X₁2E, F4I and N6T in CDR-H3; D15E, X₁16A in CDR-H2, X₁2E, F4I and N6T in CDR-H3.

5. A polypeptide or polypeptide construct comprising: a binding domain binding to an extracellular epitope of the human CD3ε chain comprising or consisting of a VH region and a VL region, wherein

- i) the VH region comprises the sequence of EVX₁LX₂ESGGGLX₃QPX₄GSLKLSCAASGFTFNX₅YAX₆NWVRQAPGKGLEWVARIRSKYNNYATYYADX₇VKX₈RFTISRDDSX₉X₁₀X₁₁X₁₂YLMNNLKTEDTAX₁₃YYCVRHX₁₄NFGNSYX₁₅SX₁₆X₁₇AYWGQGTTLVTVSX₁₈, where X₁ is Q or K; X₂ is V or L; X₃ is V or E; X₄ is G or K; X₅ is K, V, S, G, R, T, or I; X₆ is M or I; X₇ is S or Q; X₈ is D, G, K, S, or E; X₉ is K or Q; X₁₀ is N or S; X₁₁ is T or I; X₁₂ is A or L; X₁₃ is V or M; X₁₄ is G, R or A; X₁₅ is I, L, V, or T; X₁₆ is Y, W or F; X₁₇ is W, F or Y; and X₁₈ is S or A; and
- ii) the VL region comprises the sequence of QX₁VVTQEX₂X₃LTX₄SPGX₅TVTLTCX₆SSTGAVTX₇X₈X₉YX₁₀NWVQX₁₁KPX₁₂X₁₃X₁₄X₁₅X₁₆GLIGX₁₇TX₁₈X₁₉X₂₀X₂₁X₂₂GX₂₃PARFSGSLX₂₄GX₂₅KAALTX₂₆X₂₇GX₂₈QX₂₉EDEAX₃₀YX₃₁CX₃₂LWYSNX₃₃WVFGGGTKLTVL, where X₁ is T or A; X₂ is P or S; X₃ is S or A; X₄ is V or T; X₅ is G or E; X₆ is G, R, or A; X₇ is S or T; X₈ is G or S; X₉ is N or Y; X₁₀ is P or A; X₁₁ is Q or E; X₁₂ is G or D; X₁₃ is Q or H; X₁₄ is A or L; X₁₅ is P or F; X₁₆ is R or T; X₁₇ is G or A; X₁₈ is K or D; X₁₉ is F or M; X₂₀ is L or R; X₂₁ is

A, P or V; X₂₂ is P or S; X₂₃ is T or V; X₂₄ is L or I; X₂₅ is G or D; X₂₆ is L or I; X₂₇ is S or T; X₂₈ is V or A; X₂₉ is P or T; X₃₀ is E or I; X₃₁ is Y or F; X₃₂ is V, A, or T; X₃₃ is R or L; and

iii) wherein the VH and/or VL region sequence comprises one amino acid substitution or a combination thereof selected from

- a. N30, X₆34V, X₆34F, Q39, L45, D64, X₇65A, X₁₂81V, X₁₂81T, X₁₂81I, V99, H101, X₁₄102E, F104, and N106 in the VH region sequence in i); and
- b. L20, V38, X₁₁40R, X₁₁40K, X₂₄69, X₃₂91L, X₃₃93, and G102 in the VL region sequence in ii).

6. The polypeptide or polypeptide construct of claim 5, wherein in addition to said one amino acid substitution or a combination thereof as defined in iii) X₆ is I; X₈ is G; X₁₂ is L, X₁₄ is A, X₁₇ is F in said VH region sequence; and/or X₃₂ is A in said VL region sequence.

7. The polypeptide or polypeptide construct of claim 5 or 6, comprising combinations of said amino acid substitutions as defined in iii) of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more amino acid substitutions.

8. The polypeptide or polypeptide construct of any one of claims 5 to 7, wherein:

i) said one acid substitution is selected from

- a. X₆34V, Q39E, Q39K, Q39R, Q39D, L45M, L45V, X₁₂81V, X₁₂81T, X₁₂81I, V99A, V99L, H101A, H101N in the VH region; and
- b. V38I, V38L, V38M, V38F, V38Y, X₁₁40R, X₁₁40K, X₂₄69S, X₂₄69E, X₃₂91L, X₃₃93Y;

ii) said combination of two or more amino acid substitutions are selected from X₆34F and X₁₂81V in the VH region; Q39E in the VH region and X₁₁40K in the VL region; Q39E in the VH region and X₁₁40R in the VL region; Q39D in the VH region and X₁₁40K in the VL region; Q39D in the VH region and X₁₁40R in the VL region; L45V in the VH region and V38F in the VL region; L45V in the VH region and V38Y in the VL region; L45M in the VH region and X₁₁40K in the VL region; L20I and G102S in the VL region; X₁₂81V in the VH region and X₁₁40K in the VL region;

iii) said combination of three or more amino acid substitutions are selected from Q39E in the VH region, X₁₁40K and G102S in the VL region; Q39E in the VH region, X₁₁40R and G102S in the VL region; Q39D in the VH region, X₁₁40K and G102S in the VL region; Q39D in the VH region, X₁₁40R and G102S in the VL region; L45M in the VH region, X₁₁40K and G102S in the VL region; X₇65A, H101A, and N106S in the VH region; L20I, X₁₁40K, and G102S in the VL region;

- iv) said combination of four or more amino acid substitutions are selected from Q39E in the VH region, L20I, X₁₁40K and G102S in the VL region; Q39E in the VH region, L20I, X₁₁40R and G102S in the VL region; Q39D in the VH region, L20I, X₁₁40K and G102S in the VL region; Q39D in the VH region, L20I, X₁₁40R and G102S in the VL region; L45M in the VH region, L20I, X₁₁40K and G102S in the VL region; L20I, X₁₁40K, X₂₄69S, and G102S in the VL region; X₁₂81V in the VH region, L20I, X₁₁40K, and G102S in the VL region;
- v) said combination of five, six, seven, eight, nine, ten, or more amino acid substitutions are selected from X₇65A, X₁₂81V, V99A, N106S in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region; D64E, X₇65A, X₁₂81V, X₁₄102E, F104I, N106T in the VH region, L20I, X₂₄69S, and G102S in the VL region; L45M, X₇65A, N106T in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region; X₇65A, X₁₂81V, V99A, H101A, F104I, N106S in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region; X₇65A, X₁₂81V, V99A, H101A, N106S in the VH region; X₁₂81V, V99A in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region; Q39E, X₁₂81V in the VH region, L20I, X₁₁40K, and G102S in the VL region; L45V in the VH region, L20I, V38F, X₁₁40K and G102S in the VL region.
9. The polypeptide or polypeptide construct of any one of claims 5 to 8, wherein said combination of amino acid substitutions is selected from:
- i) in the VH region
- X₇65A, X₁₂81V, V99A, H101A and N106S;
 - D64E, X₇65A, A81V, H101N, X₁₄102E, F104I, and N106S;
 - L45M, X₇65A, H101A, and N106T;
 - L45M, X₇65A, H101A, and N106S;
 - Q39E, X₇65A, H101N, and N106T;
 - D64E, X₇65A, V99A, H101A, and N106T;
 - X₇65A, X₁₂81V, V99A, H101A, X₁₄102E, F104I, and N106T;
 - X₇65A, X₁₂81V, H101N, X₁₄102E, F104I, and N106S;
 - D64E, X₇65A, X₁₂81V, H101A, and N106S;
 - D64E, X₇65A, H101A, and N106T;
 - X₇65A, V99A, H101A, and N106T;
 - D64E, X₇65A, H101A, and N106S;
 - D64E, X₇65A, X₁₂81V, V99A, H101A, and N106S;
 - X₇65A, H101A and N106S;
 - N30S, Q39E, D64E, X₇65A, X₁₂81V, H101A, X₁₄102E, F104I, and N106T;

- p. L45M, D64E, X₇65A, H101A, and N106T;
 - q. N30S, L45M, X₇65A, X₁₂81V, H101A, and N106T;
 - r. N30S, L45M, D64E, X₇65A, X₁₂81V, H101A, and N106S;
- ii) in the VL region
- a. L20I, X₁₁40K, X₂₄69S, and G102S;
 - b. L20I, X₂₄69S, and G102S;
 - c. L20I, V38I, X₁₁40K, X₂₄69E, G102S and X₃₃93Y;
 - d. X₁₁40K and G102S;
 - e. L20I, X₁₁40K, X₂₄69S, G102S and X₃₃93Y;
 - f. L20M, X₁₁40K and X₂₄69E;
 - g. L20I, V38I, X₁₁40K, X₂₄69E and G102S;
 - h. X₁₁40K, X₂₄69S and X₃₃93Y; or
 - i. X₁₁40K and X₂₄69S; and
- iii) a combination of one amino acid substitution combination of i) and ii).
10. The polypeptide or polypeptide construct of claim 9, wherein said combination of amino acid substitution combinations of iii) is selected from:
- a. X₇65A, X₁₂81V, V99A, H101A, N106S in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region;
 - b. D64E, X₇65A, X₁₂81V, H101N, X₁₄102E, F104I, N106S in the VH region, L20I, X₂₄69S, and G102S in the VL region;
 - c. L45M, X₇65A, H101A, N106T in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region;
 - d. L45M, X₇65A, H101A, N106S in the VH region, L20I, V38I, X₁₁40K, X₂₄69E, G102S and X₃₃93Y in the VL region;
 - e. Q39E, X₇65A, H101N, N106T in the VH region, X₁₁40K and G102S in the VL region;
 - f. D64E, X₇65A, V99A, H101A, N106T in the VH region, X₁₁40K and G102S in the VL region;
 - g. X₇65A, X₁₂81V, V99A, H101A, X₁₄102E, F104I, N106T in the VH region, L20I, X₁₁40K, X₂₄69S, and G102S in the VL region;
 - h. X₇65A, X₁₂81V, H101N, X₁₄102E, F104I, N106S in the VH region, L20I, X₂₄69S, and G102S in the VL region;
 - i. D64E, X₇65A, X₁₂81V, H101A, N106S in the VH region, L20I, X₂₄69S, and G102S in the VL region;
 - j. D64E, X₇65A, H101A, N106T in the VH region, X₁₁40K and G102S in the VL region;

- k. X₇65A, V99A, H101A, N106T in the VH region, X₁₁40K and G102S in the VL region;
- l. D64E, X₇65A, H101A, N106S in the VH region, X₁₁40K and G102S in the VL region;
- 5 m. D64E, X₇65A, X₁₂81V, V99A, H101A, N106S in the VH region, L20I, X₁₁40K, X₂₄69S, G102S and X₃₃93Y in the VL region;
- n. X₇65A, H101A, N106S in the VH region, X₁₁40K and G102S in the VL region;
- o. N30S, Q39E, D64E, X₇65A, X₁₂81V, H101A, X₁₄102E, F104I, N106T in the VH region, L20M, X₁₁40K and X₂₄69E in the VL region;
- 10 p. L45M, D64E, X₇65A, H101A, N106T in the VH region, L20I, V38I, X₁₁40K, X₂₄69E and G102S in the VL region;
- q. N30S, L45M, X₇65A, X₁₂81V, H101A, N106T in the VH region, X₁₁40K and X₂₄69S in the VL region; and
- r. N30S, L45M, D64E, X₇65A, X₁₂81V, H101A, N106S in the VH region, X₁₁40K and
15 X₂₄69S in the VL region.

11. The polypeptide or polypeptide construct of claim 10, wherein in said combination of amino acid substitution combinations defined in:
- a. and m. X₆ is I in said VH region sequence;
 - 20 b. and r. X₈ is G in said VH region sequence;
 - c. X₁₂ is L in said VH region;
 - d. X₁₂ is L and X₁₇ is F in said VH region;
 - e. X₆ is I, X₁₄ is A and X₁₇ is F in said VH region sequence;
 - f. X₅ is I in said VH region sequence; and X₃₂ is A in said VL region sequence;
 - 25 g. X₅ is I in said VH region sequence;
 - h. and i. X₈ is G in said VH region sequence;
 - j., l. and n. X₆ is I and X₁₇ is F in said VH region sequence; and X₃₂ is A in said VL region sequence;
 - k. X₆ is I said VH region sequence; and X₃₂ is A in said VL region sequence;
 - 30 p. X₁₂ is L in said VH region; and
 - q. X₈ is G and X₁₇ is F in said VH region sequence.

12. The polypeptide or polypeptide construct of claim 5, or any one of claims 6 to 11, wherein said VH and VL region of i) and ii) is selected from the VH and VL region combinations as defined in SEQ ID NOs: 124 and 125, 172 and 173, 362 and 363, 552 and 553, 730 and 731, 928 and 929, 1106 and 1107, 1296 and 1297, 1474 and 1475, 1664 and 1665, and 1854 and 1855, wherein said one amino acid substitution or a
35

combination thereof in said VH and/or VL region sequence results in a VH and/or a VL region sequence having the amino acid residue:

- i.
- 5 I at position 34, A at position 65, V at position 81, A at position 99, A at position 101, S at position 106 in the VH region sequence, I at position 20, K at position 40, S at position 69, and S at position 102 in the VL region sequence;
- M at position 45, A at position 65, L at position 81, A at position 101, T at position 106 in the VH region sequence, I at position 20, K at position 40, S at position 69 and S at position 102 in the VL region sequence;
- 10 M at position 45, A at position 65, L at position 81, A at position 101, S at position 106, F at position 112 in the VH region sequence, I at position 20, I at position 38, K at position 40, E at position 69, S at position 102 and Y at position 93 in the VL region sequence;
- I at position 34, E at position 39, A at position 65, N at position 101, A at position 102, T at position 106, F at position 112 in the VH region sequence, K at position 40 and S at position 102 in the VL region sequence;
- 15 I at position 34, E at position 64, A at position 65, A at position 99, A at position 101, T at position 106 in the VH region sequence, K at position 40, A at position 91 and S at position 102 in the VL region sequence;
- I at position 34, A at position 65, V at position 81, A at position 99, A at position 101, E at position 102, I at position 104, T at position 106 in the VH region sequence, I at position 20, K at position 40, S at position 69 and S at position 102 in the VL region sequence;
- 20 E at position 64, A at position 65, G at position 68, V at position 81, A at position 101, S at position 106 in the VH region sequence, I at position 20, S at position 69 and S at position 102 in the VL region sequence;
- 25 I at position 34, E at position 64, A at position 65, A at position 101, T at position 106, F at position 112 in the VH region sequence, K at position 40, A at position 91 and S at position 102 in the VL region sequence;
- I at position 34, E at position 64, A at position 65, A at position 101, S at position 106, F at position 112 in the VH region sequence, K at position 40, A at position 91 and S at position 102 in the VL region sequence;
- 30 I at position 34, E at position 64, A at position 65, V at position 81, A at position 99, A at position 101, S at position 106 in the VH region sequence, I at position 20, K at position 40, S at position 69, S at position 102 and Y at position 93 in the VL region sequence;
- 35 I at position 34, A at position 65, A at position 101, S at position 106, F at position 112 in the VH region sequence, K at position 40, A at position 91 and S

at position 102 in the VL region sequence;
M at position 45, E at position 64, A at position 65, L at position 81, A at position 101, T at position 106 in the VH region sequence, I at position 20, I at position 38, K at position 40, E at position 69 and S at position 102 in the VL region sequence;
5 S at position 30, M at position 45, A at position 65, G at position 68, V at position 81, A at position 101, T at position 106, F at position 112 in the VH region sequence, K at position 40, S at position 69 and Y at position 93 in the VL region sequence; and
10 S at position 30, M at position 45, E at position 64, A at position 65, G at position 68, V at position 81, A at position 101, S at position 106 in the VH region sequence, K at position 40 and S at position 69 in the VL region sequence;
ii. E at position 64, A at position 65, G at position 68, V at position 81, N at position 101, E at position 102, I at position 104, T at position 106 in the VH region sequence, I at position 20, S at position 69 and S at position 102 in the VL region sequence;
15 A at position 65, G at position 68, V at position 81, N at position 101, E at position 102, I at position 104, S at position 106 in the VH region sequence, I at position 20, S at position 69 and S at position 102 in the VL region sequence;
20 I at position 34, A at position 65, A at position 99, A at position 101, T at position 106 in the VH region sequence, K at position 40, A at position 91 and S at position 102 in the VL region sequence; and
S at position 30, E at position 39, E at position 64, A at position 65, V at position 81, A at position 101, E at position 102, I at position 104, T at position 106 in the VH region sequence, M at position 20, K at position 40 and E at position 69 in the VL region sequence;
25
iii. A at position 101 in the VH region sequence;
iv. V at position 81, A at position 99 in the VH region sequence, I at position 20, K at position 40, S at position 69 and S at position 102 in the VL region sequence;
30 I at position 34, V at position 81 in the VH region sequence, I at position 20, K at position 40 and S at position 102 in the VL region sequence;
I at position 34 in the VH region sequence, I at position 20, K at position 40 and S at position 102 in the VL region sequence;
35 I at position 34, E at position 39, V at position 81 in the VH region sequence, I at position 20, K at position 40 and S at position 102 in the VL region sequence;

- v. I at position 34, V at position 81 in the VH region sequence, and K at position 40 in the VL region sequence;
M at position 45 in the VH region sequence, I at position 20, K at position 40 and S at position 102 in the VH region sequence;
5 I at position 34, A at position 65, A at position 101 and S at position 106S in the VH region sequence;
I at position 20, K at position 40, S at position 69 and S at position 102 in the VL region sequence;
F at position 34 and V at position 81 in the VH region sequence;
10 M at position 45 in the VH region sequence, K at position 40 and S at position 102 in the VL region sequence;
D at position 39 in the VH region sequence, I at position 20, K at position 40 and S at position 102 in the VL region sequence;
I at position 34 and V at position 81 in the VH region sequence;
15 E at position 39 in the VH region sequence, I at position 20, K at position 40 and S at position 102 in the VL region sequence;
M at position 45 in the VH region sequence and K at position 40 in the VL region sequence;
I at position 34, A at position 65, V at position 81, A at position 99, A at position 101 and S at position 106 in the VH region sequence; or
20 vi. D at position 39 in the VH region sequence, I at position 20, R at position 40 and S at position 102 in the VL region sequence;
I at position 34 in the VH region sequence;
K at position 40 in the VL region sequence;
25 D at position 39 in the VH region sequence, K at position 40 and S at position 102 in the VL region sequence;
I at position 20 and S at position 102 in the VH region sequence;
K at position 39 in the VH region sequence;
E at position 39 in the VH region sequence, K at position 40 and S at position 30 102 in the VL region sequence;
E at position 39 in the VH region sequence, I at position 20, R at position 40 and S at position 102 in the VL region sequence;
D at position 39 in the VH region sequence, R at position 40 and S at position 102 in the VL region sequence;
35 V at position 81 in the VH region sequence;
D at position 39 in the VH region sequence and K at position 40 in the VL region sequence;

- F at position 112 in the VH region sequence;
F at position 112 in the VH region sequence and_I at position 38 in the VL region sequence;
E at position 39 in the VH region sequence and K at position 40 in the VL region sequence;
V at position 34 in the VH region sequence.
13. The polypeptide or polypeptide construct of any one of claims 1 to 12, wherein the VH and VL region sequence are linked by a linker.
14. The polypeptide or polypeptide construct of claim 13, wherein the linker is a peptide linker.
15. The polypeptide or polypeptide construct of claim 14, wherein said peptide linker comprises or consists of a G₄S linker, or G₄Q linker or repetitions thereof.
16. The polypeptide or polypeptide construct of any one of claims 1 to 15, wherein the polypeptide or polypeptide construct comprises at least one further binding domain.
17. The polypeptide or polypeptide construct of claim 16, wherein said at least one further binding domain binds to a cell surface antigen.
18. The polypeptide or polypeptide construct of claim 17, wherein said cell surface antigen is a tumor antigen.
19. The polypeptide or polypeptide construct of claim 18, wherein the tumor antigen is selected from the group consisting of BCMA, CD123, CD19, CD20, CD22, CD33, CD70, CDH19, CDH3, CLL1, CS1, CLDN6, CLDN18.2, DLL3, EGFRvIII, FLT3, MAGEB2, MART1, MSLN, MUC17, PSMA, and STEAP1.
20. The polypeptide or polypeptide construct of any one of claims 1 to 19, wherein said polypeptide or polypeptide construct is a single chain polypeptide that is at least bispecific.
21. A polynucleotide encoding a polypeptide or polypeptide construct as defined in any one of claims 1 to 20.

22. A vector comprising a polynucleotide as defined in claim 21.
23. A host cell transformed or transfected with the polynucleotide as defined in claim 21 or with the vector as defined in claim 22.
- 5 24. A process for the production of an polypeptide or polypeptide construct according to any one of claims 1 to 20, said process comprising culturing a host cell as defined in claim 23 under conditions allowing the expression of the polypeptide or polypeptide construct as defined in any one of claims 1 to 20 and recovering the produced polypeptide or
- 10 polypeptide construct from the culture.
25. A pharmaceutical composition comprising a polypeptide or polypeptide construct according to any one of claims 1 to 20, or produced according to the process of claim 24.
- 15 26. The polypeptide or polypeptide construct of any one of claims 18 to 20, or the polypeptide or polypeptide construct produced according to the process of claim 24, for use in therapy~~the prevention, treatment or amelioration of a disease selected from a tumorous disease.~~
- 20 27. Use of the polypeptide or polypeptide construct according to any one of claims 18 to 20, or the polypeptide or polypeptide construct produced according to the process of claim 24 in the manufacture of a medicament ~~A method for the prevention, treatment or amelioration of a tumorous disease, comprising administering to a subject in need thereof the polypeptide or polypeptide construct according to any one of claims 18 to 20, or~~
- 25 ~~produced according to the process of claim 24.~~
28. A kit comprising a polypeptide or polypeptide construct according to any one of claims 1 to 20, or produced according to the process of claim 24, a polynucleotide as defined in claim 21, a vector as defined in claim 22, and/or a host cell as defined in claim 23.
- 30