Claims

1. A hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof comprising:

a. a first engineered immunoglobulin chain comprising an engineered parent domain having a protein-protein interface, wherein at least one amino acid residue of the protein-protein interface of the parent domain of said first engineered immunoglobulin chain is substituted with an amino acid residue from a protein-protein interface of a first donor domain; and

b. a second engineered immunoglobulin chain comprising an engineered parent domain having a protein-protein interface, wherein at least one amino acid residue of the protein-protein interface of the parent domain of said second engineered immunoglobulin chain is substituted with an amino acid residue from a protein-protein interface of a second donor domain, wherein the engineered parent domain of the first engineered immunoglobulin chain and the engineered parent domain of the second engineered immunoglobulin chain are not identical and, wherein the first donor domain and the second donor domain form a naturally occurring TCR constant domain hetero-dimer or a naturally occurring TCR constant domain homo-dimer and, wherein the parent domain of the first and/or the second engineered immunoglobulin chain is a CH3 domain, wherein the amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain comprises the amino acid residue at position 88, and, wherein the amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain comprises the amino acid residue at position 85.1 and/or 86, and wherein the amino acid residue substituted at position 88 in the parent domain of the first engineered immune-globulin chain is interacting with the amino acid residue substituted at position 85.1 and/or 86 in the parent domain of the second engineered immunoglobulin chain, wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

2. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein the parent domain of the first and/or the second engineered immunoglobulin chain is a naturally occurring CH3 domain.

3. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain

comprise a third engineered domain with a protein-protein interface, wherein at least one amino acid residue of the protein-protein interface of the parent domain of said further engineered domain of said first engineered immunoglobulin chain and/or said second engineered immunoglobulin chain is substituted with an amino acid residue from a protein-protein interface of a third donor domain, and wherein the protein-protein interface of the further engineered domain of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain is interacting with the protein-protein interface of an engineered domain of a third engineered immunoglobulin chain by hetero-dimerization or by homodimerization, wherein at least one amino acid residue of the protein-protein interface of the parent domain of said engineered domain of said third engineered immunoglobulin chain is substituted with an amino acid residue from a protein-protein interface of a fourth donor domain, and, wherein the third donor domain and the fourth donor domain form a naturally occurring TCR constant domain hetero-dimer or a naturally occurring TCR constant domain homo-dimer and, wherein the protein-protein interface of the further engineered domain of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain and of the engineered domain of the third engineered immunoglobulin chain is different from the protein-protein interface of the engineered domain of the first engineered immunoglobulin chain and of the engineered domain of the second engineered immuno- globulin chain.

4. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein the first and second engineered immunoglobulin chains comprise an Fe region.

5. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 3, wherein at least two amino acid residues of the protein-protein interface of the parent domain of the further engineered domain of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain and/or of the parent domain of the engineered domain of the third engineered immunoglobulin chain are substituted.

6. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 3, wherein the hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof or the full-length antibody is bispecific.

7. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 6, wherein the bispecific heterodimeric immunoglobulin or hetero-dimeric fragment thereof or the bispecific

full-length antibody binds to antigens selected from the group consisting ofHER2, EGFR, CD19, and VLA-2.

8. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein at least one additional polypeptide is fused to the first and/or second engineered immunoglobulin chain.

9. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 8, wherein said additional polypeptide is selected from the group consisting of Fab, scFv, diabody, domain antibody, pharmacologically active peptide or protein, receptor extracellular domain, CDR grafted polypeptide, and therapeutic engineered protein scaffold.

10. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein the amino acid residues of the protein-protein interface of the parent domain of the first and/or second engineered immunoglobulin chain which are substituted are not adjacent.

11. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof claim 1, wherein at least two amino acid residues of the protein-protein interface of the parent domain of the first and/or second engineered immunoglobulin chain are substituted.

12. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof claim 1, wherein the parent domain of the first engineered immunoglobulin chain is an IgGl CH3 domain and the parent domain of the second engineered immunoglobulin chain is an IgG3 CH3 domain or wherein the parent domain of the first engineered immunoglobulin chain is an IgG3 CH3 domain and the parent domain of the second engineered immunoglobulin chain is an IgG 1 CH3 domain.

13. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein the amino acid sequence of the engineered domains of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain do not contain an insertion of one or more amino acid residues compared to the amino acid sequence of the parent domains of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain.

14. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein a further amino acid residue at a position selected from the group consisting of 3, 5, 7, 20, 22, 26,

27, 79, 81, 84, 84.2, 85.1, 86, and 90 is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain.

15. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, wherein a further amino acid residue at a position selected from the group consisting of 3, 5, 7, 20, 22, 26, 27, 79, 81, 84, 84.2, 85.1, 86, 88, and 90 is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain.

16. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 14 or claim 15, wherein:

a) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain at position 88 is 88W and conservative amino acid substitutions thereof, wherein the amino acid position is indicated according to the IMGT® numbering;

b) the amino acid residue which is substituted at position 85 and/or 86 in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain is selected from the group consisting of 85.lA, 85.lS, 85.lC and 85.lN and conservative amino acid substitutions thereof and/or is selected from the group consisting of 86S and 86V and conservative amino acid substitutions thereof, wherein the amino acid position is indicated according to the IMGT® numbering;

c) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain at position 88 is selected from the group consisting of 88Q, SSL, 88V, 88R, SSE, 88I, 88T, 88Y, 88K and 88W and/or wherein the further amino acid residue substituted in the protein- protein interface of the parent domain of the first engineered immunoglobulin chain is selected from the group consisting of 20\7, 20T, 20A, 20N, 20Q, 20E, 20S, 20K, 20W, 22A, 22G, 22T, 22L, 22I, 22V, 26R, 26Q, 26T, 26K, 26\7, 26S, 26N, 26E, 85.lT, 85.lM, 85.lA, 85.lS, 85.lR, 85.lH, 85.lK, 85.lF, 85.lC, 85.lN, 85.lW, 86S, 86I, 86T, 86H, 86Q, 86\7, 86W, 86Y and 86F, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

d) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain at position 88 is selected from the group

consisting of 88Q, SSL, 88V, 88R, SSE, 88I, 88T, 88Y, and 88W and/or wherein the further amino acid residue substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is selected from the group consisting of 20\7, 20T, 20A, 20N, 20Q, 20E, 20S, 20K, 22A, 22G, 22T, 22L, 22I, 22\7, 26Q, 26T, 26K, 26\7, 26S, 26N, 26E, 85.lT, 85.lM, 85.lA, 85.lS, 85.lR, 85.lH, 85.lK, 85.lF, 85.lC, 85.lN, 86S, 86I, 86T, 86H, 86Q, 86\7, and 86F, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

e) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain comprises the amino acid residues at position 88 and at position 20, and optionally a further amino acid residue at a position selected from the group consisting of 3, 5, 7, 22, 26, 27,79, 81, 84, 84.2, 85.1, 86, and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

f) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain comprises the amino acid residues at position 85.1 and/or 86 and at position 26 and optionally an amino acid residue at a further position, wherein the amino acid position is indicated according to the IMGT® numbering;

g) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain comprises 15 the amino acid residues at position 85 .1 and 86 or comprises 85.lC and conservative amino acid substitutions thereof and 86S and conservative amino acid substitutions thereof, and wherein the further amino acid residue which is substituted in the protein-protein 20 interface of the parent domain of the second engineered immunoglobulin chain is selected from the group consisting of3E, SA, 7F, 20T, 22\7, 26T, 81D, 84L, 84.2E,88R and 90R and conservative amino acid substitutions of any of these thereof, wherein the amino acid position 25 of each group member is indicated according to the IMGT® numbering;

h) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain comprises 30 the amino acid residues at position 85 .1 and 86 or comprises 85.lN and conservative amino acid substitutions thereof and 86V and conservative amino acid substitutions thereof, and wherein the further amino acid residue which is substituted in the protein-protein 35 interface of the parent domain of the second

engineered immunoglobulin chain is selected from the group consisting of7M, 20N, 22A, 27E, 79F, 81A, 84.2S, 85.lN, 86V, SSL, and 90K and conservative amino acid substitutions of any of these thereof, wherein the amino 40 acid position of each group member is indicated according to the IMGT® numbering; or

i) the amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is 88I and conservative amino acid substitutions thereof and the optional further amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is 81 Wand conservative amino acid substitutions thereof, wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

17. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 14 or claim 15, wherein:

a) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is selected from the group consisting of 3E, 3K, SA, ST, 7F, 7M, 20K, 20N, 20T, 22A, 22L, 22\7, 26E, 26T, 27E, 27K, 79F, 79Y, 81A, 81G, 81D, 84L, 84M, 84.2E, 84.2S, 85.lA, 85.lC, 85.lM, 85.lN, 85.lS, 86F, 86S, 86\7, 90K, 90N, and 90R and conservative amino acid substitutions of any of these thereof, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

b) wherein the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is at a position selected from the group consisting of 20, 22, 26, 79, 85.1, 86, and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

c) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is selected from the group consisting of 20K, 22\7, 26T, 79Y, 85.lS, 86\7, and 90N and conservative amino acid substitutions of any of these thereof, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

d) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain is at position 84 or is 84L and conservative amino acid substitutions thereof, and optionally a further amino acid at a position selected from the group consisting of 3, 5, 7, 20, 22, 26, 81, 84.2, 85.1, 86, 88, and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

e) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain is at a position selected from the group consisting of 3, 5, 7, 20, 22, 26, 81, 84, 84.2, 85.1, 86, 88, and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

f) wherein the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is at a position selected from the group consisting of 3, 5, 20, 22, 26, 27, 81, 84, 85.1, and 86, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

g) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the first engineered immunoglobulin chain is selected from the group consisting of 3K, 5T, 20T, 22L, 26E, 27K, 81G, 84M, 85.lM, 86F and conservative amino acid substitutions of any of these thereof, wherein the amino acid position of each group member is indicated according to the IMGT® numbering;

h) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain is at a position selected from the group consisting of7, 20, 22, 27, 79, 81, 84.2, 85.1, 86, 88, and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering; or

i) the further amino acid residue which is substituted in the protein-protein interface of the parent domain of the second engineered immunoglobulin chain is at position 79 or is 79F and conservative amino acid substitutions thereof, and optionally a further amino acid residue at a position selected from the group consisting of 7, 20, 22, 27, 81, 84.2, 85.1, 86, 88, and 90, wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

18. The hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 17, wherein the amino acid residue substitution 85 .1 C is replaced by amino acid residue substitution 85.lAor 85.lS, wherein the amino acid position of each group member is indicated according to the IMGT® numbering.

19. A method of producing a hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof of claim 1, the method comprising:

(a) substituting at least one amino acid residue of the protein-protein interface of a parent domain of a first parent immunoglobulin chain with a residue from a protein-protein interface of a first donor domain to obtain a first engineered immunoglobulin chain comprising an engineered domain;

(b) substituting at least one amino acid residue of the protein-protein interface of the parent domain of a second parent immunoglobulin chain with a residue from a protein-protein interface of a second donor

domain to obtain a second engineered immunoglobulin chain comprising an engineered domain;

(c) culturing a host cell comprising a nucleic acid encoding said engineered immunoglobulin chains, wherein the culturing is such that the nucleic acid is expressed and the engineered immunoglobulin chains produced; and

(d) recovering the hetero-dimeric immunoglobulin or hetero-dimeric fragment thereof from the host cell culture.

20. The method of claim 19, wherein:

a) the first donor domain is the TCR constant domain alpha (SEQ ID NO: 1) and the second donor domain is the TCR constant domain beta (SEQ ID NO: 2);

b) the first donor domain is the TCR constant domain alpha (SEQ ID NO: 1) and the second donor domain is the TCR constant domain beta (SEQ ID NO: 2), wherein the cysteine (C) at amino acid position 75 in SEQ ID NO: 2 is substituted with alanine (A) or serine (S); or

c) the first donor domain is the TCR constant domain gamma (SEQ ID NO: 33) and second donor domain is the TCR constant domain delta (SEQ ID NO: 32).

21. The method of claim 19, wherein the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain comprise a further engineered domain with a protein-protein interface, wherein at least one amino acid residue of the protein-protein interface of the parent domain of said further engineered domain of said first engineered immunoglobulin chain and/or said second engineered immunoglobulin chain is substituted with an amino acid residue from a protein-protein interface of a third donor domain, and wherein the protein-protein interface of the further engineered domain of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain is interacting with the protein-protein interface of an engineered domain of a third engineered immunoglobulin chain by hetero-dimerization or by homodimerization, wherein at least one amino acid residue of the protein-protein interface of the parent domain of said engineered domain of said third engineered immunoglobulin chain is substituted with an amino acid residue from a protein-protein interface of a fourth donor domain and, wherein the protein-protein interface of the further engineered domain of the first engineered immunoglobulin chain and/or the second engineered immunoglobulin chain and of the engineered domain of the third engineered immunoglobulin chain is different from the protein-protein interface of the engineered domain of the first engineered immunoglobulin chain and of the engineered domain of the second engineered immunoglobulin chain.