

generation for clinical hetero-mAb therapeutics (Biotechnol. Prog. 33 (2017) 469-477).

Bahr et al. reported the development of a platform expression system using targeted integration in Chinese hamster ovary cells (proceedings of Cell Culture Engineering XVI, 2018).

Carver et al. reported maximizing antibody production in a targeted integration host by optimization of subunit gene dosage and position (Biotechnol. Prog. (2020) e2967).

Summary of the Invention

The current ~~invention~~disclosure is defined by the following independent aspects and dependent ~~embodiment~~examples:

1. A first aspect of the ~~invention~~disclosure is a method for producing an antibody-multimer-fusion polypeptide

comprising

- (a) an antibody heavy chain and an antibody light chain, and
- (b) a first fusion polypeptide comprising in N- to C-terminal direction a first part of a non-antibody multimeric polypeptide, an antibody heavy chain CH1 domain or an antibody light chain constant domain, an antibody hinge region, an antibody heavy chain CH2 domain and an antibody heavy chain CH3 domain, and a second fusion polypeptide comprising in N- to C-terminal direction the second part of the non-antibody multimeric polypeptide and an antibody light chain constant domain if the first polypeptide comprises an antibody heavy chain CH1 domain or an antibody heavy chain CH1 domain if the first polypeptide comprises an antibody light chain constant domain,

wherein

- (i) the antibody heavy chain of (a) and the first fusion polypeptide of (b), (ii) the antibody heavy chain of (a) and the antibody light

chain of (a), and (iii) the first fusion polypeptide of (b) and the second fusion polypeptide of (b) are each independently of each other covalently linked to each other by at least one disulfide bond,

wherein

the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to an antigen, characterized in that the antibody-multimer-fusion polypeptide is expressed by a recombinant mammalian cell obtained by transfecting a (parent) mammalian cell with the expression cassettes for the antibody heavy chain, the antibody light chain, the first fusion polypeptide and the second fusion polypeptide at a stoichiometric ratio of 1:1:2:1.

2. The method ~~according to aspect 1 as described above~~, wherein the antibody-multimer-fusion polypeptide is transiently or stably expressed.
3. The method ~~according to any one of aspect 1 or embodiment 2 as described above~~, wherein the mammalian cell is a CHO cell, preferably a CHO-K1, or a HEK cell.
4. The method ~~according to any one of aspect 1 and embodiments 2 to 3 as described above~~, wherein the transfecting is of four vectors, whereby each vector comprises exactly one of the expression cassettes.
5. The method ~~according to any one of aspect 1 and embodiments 2 to 3 as described above~~, wherein the transfecting is of three vectors, whereby two vectors comprise exactly two of the expression cassettes and one vector comprises exactly one of the expression cassettes.
6. The method ~~according to embodiment 4 as described above~~, wherein the transfecting is of three vectors, whereby the first vector comprises the expression cassettes for the an antibody heavy chain and an antibody light chain, the second expression vector comprises the expression cassettes for the first fusion polypeptide and the second fusion polypeptide, and the third vector comprises one expression cassette for the first fusion polypeptide.
7. The method ~~according to any one of aspect 1 and embodiments 2 to 6 as described above~~, wherein the first fusion polypeptide comprises as first part of the non-antibody

multimeric polypeptide two ectodomains of a TNF ligand family member or a fragment thereof that are connected to each other by a peptide linker, and the second fusion polypeptide comprises as second part of a non-antibody multimeric polypeptide only one ectodomain of said TNF ligand family member or a fragment thereof, or vice versa.

8. The method ~~according to embodiment 7~~ as described above, wherein
 - (a) the first fusion polypeptide comprises as first part of the non-antibody multimeric polypeptide a first ectodomain of a TNF ligand family member or a fragment thereof, a spacer domain and a second ectodomain of said TNF ligand family member or a fragment thereof, wherein
 - the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
 - the first ectodomain of a TNF ligand family member or a fragment thereof is fused either directly or via a first peptide linker to the N-terminus of the spacer domain and
 - the second ectodomain of said TNF ligand family member or a fragment thereof is fused either directly or via a second peptide linker to the C-terminus of the spacer domain,
 - (b) the second fusion polypeptide comprises as second part of the non-antibody multimeric polypeptide a third ectodomain of said TNF ligand family member or a fragment thereof that is fused either directly or via a third peptide linker to
 - either the C-terminus of the second ectodomain of said TNF ligand family member in the first fusion polypeptide or to the C-terminus of the spacer domain in the second fusion polypeptide, or
 - in case the second part of the antigen binding domain is fused to the C-terminus of the spacer domain of the second fusion protein, to the C-terminus of the second ectodomain of said TNF ligand family member in the first fusion polypeptide.

9. The method ~~according to any one of aspect 1 and embodiments 2 to 8~~as described above, wherein

the first fusion polypeptide comprises in N- to C-terminal direction a first part of a non-antibody multimeric polypeptide, an antibody light chain constant domain, an antibody hinge region, an antibody heavy chain CH2 domain and an antibody heavy chain CH3 domain, and

the second fusion polypeptide comprising in N- to C-terminal direction the second part of the non-antibody multimeric polypeptide and an antibody heavy chain CH1 domain.
10. The method ~~according to any one of aspect 1 and embodiments 2 to 9~~as described above, wherein

the first fusion polypeptide comprises the knob mutation, and

the antibody heavy chain comprises the hole mutations.
11. The method ~~according to any one of aspect 1 and embodiments 2 to 10~~as described above, wherein the antibody heavy chain of (a) and the first fusion polypeptide of (b) form an Fc-region.
12. The method ~~as described above~~~~according to any one of aspect 1 and embodiments 2 to 11~~, wherein the antibody heavy chain of (a) and the first fusion polypeptide of (b) form an IgG1 Fc-region or an IgG4 Fc-region.
13. The method ~~as described above~~~~according to any one of aspect 1 and embodiments 2 to 12~~, wherein the Fc-region is an IgG1 Fc-region further comprising the amino acid substitutions at positions 234 and 235 and/or 329 (Kabat EU numbering).
14. The method ~~as described above~~~~according to any one of aspect 1 and embodiments 2 to 13~~, wherein the Fc-region is an IgG1 Fc-region further comprising the amino acid substitutions L234A, L235A and/or P329G (Kabat EU numbering).
15. The method ~~as described above~~~~according to any one of embodiments 7 to 14~~, wherein in the first fusion polypeptide the two ectodomains of a TNF ligand family member or

fragments thereof connected to each other by a first peptide linker and are fused at its C-terminus by a second peptide linker to a CH1 domain, and in the second fusion polypeptide the one ectodomain of said TNF ligand family member or a fragment thereof is fused at its C-terminus by a third peptide linker to the antibody light chain constant domain.

16. The method as described above~~according to any one of embodiments 7 to 14~~, wherein in the first fusion polypeptide the two ectodomains of a TNF ligand family member or fragments thereof connected to each other by a first peptide linker and are fused at its C-terminus by a second peptide linker to light chain constant domain, and in the second fusion polypeptide the one ectodomain of said TNF ligand family member or a fragment thereof is fused at its C-terminus by a third peptide linker to the a heavy chain CH1 domain.
17. The method as described above~~according to any one of aspect 1 and embodiments 2 to 16~~, wherein in the CL domain adjacent to the part of a non-antibody multimeric polypeptide the amino acid at position 123 (Kabat EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (Kabat EU numbering) has been substituted by lysine (K), and wherein in the CH1 domain adjacent to the part of a non-antibody multimeric polypeptide the amino acids at position 147 (Kabat EU numbering) and at position 213 (Kabat EU numbering) have been substituted by glutamic acid (E).
18. The method as described above~~according to any one of aspect 1 and embodiments 2 to 17~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to a cell surface antigen.
19. The method as described above~~according to any one of aspect 1 and embodiments 2 to 18~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to a cell surface antigen selected from the group consisting of Fibroblast Activation Protein (FAP), Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Carcinoembryonic Antigen (CEA), CD19, CD20 and CD33.

20. The method as described above~~according to any one of embodiments 7 to 19~~, wherein the TNF ligand family member co-stimulates human T-cell activation.
21. The method as described above~~according to any one of embodiments 7 to 20~~, wherein the TNF ligand family member is selected from 4-1-BBL and OX40L.
22. The method as described above~~according to any one of embodiments 7 to 21~~, wherein the TNF ligand family member is 4-1-BBL.
23. The method as described above~~according to any one of embodiments 7 to 22~~, wherein the ectodomain of a TNF ligand family member comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 01, SEQ ID NO: 02, SEQ ID NO: 03, SEQ ID NO: 04, SEQ ID NO: 56, SEQ ID NO: 100, SEQ ID NO: 101 and SEQ ID NO: 102.
24. The method as described above~~according to any one of embodiments 7 to 23~~, wherein the ectodomain of a TNF ligand family member comprises the amino acid sequence of SEQ ID NO: 01 or SEQ ID NO: 56.
25. The method as described above~~according to any one of aspect 1 and embodiments 2 to 24~~, wherein
 - (a) the antibody heavy chain and the antibody light chain form a binding site capable of specific binding to a target cell antigen, and
 - (b) the first fusion polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 05, SEQ ID NO: 57, SEQ ID NO: 58 and SEQ ID NO: 59, and the second fusion polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 01, SEQ ID NO: 56, SEQ ID NO: 03 and SEQ ID NO: 04.
26. The method as described above~~according to any one of embodiments 7 to 21~~, wherein the TNF ligand family member is OX40L.
27. The method as described above~~according to any one of embodiments 7 to 21 and 26~~, wherein the ectodomain of a TNF ligand family member comprises the amino acid sequence

of SEQ ID NO: 43 or SEQ ID NO: 44, particularly the amino acid sequence of SEQ ID NO: 43.

28. The method ~~as described above~~~~according to any one of embodiments 7 to 21 and 26 to 27~~, wherein the antibody-multimer-fusion comprises
 - (a) at least one moiety capable of specific binding to a target cell antigen, and
 - (b) the first and the second fusion polypeptide are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 99 or SEQ ID: 100 and in that the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 43 or SEQ ID NO: 44.
29. The method ~~as described above~~~~according to any one of aspect 1 and embodiments 2 to 28~~, wherein the antigen is Fibroblast Activation Protein (FAP).
30. The method ~~as described above~~~~according to any one of aspect 1 and embodiments 2 to 29~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to FAP and comprise a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 06 or SEQ ID NO: 60, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 07 or SEQ ID NO: 61, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 08 or SEQ ID NO: 62, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 09 or SEQ ID NO: 63, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 64, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 65.
31. The method ~~as described above~~~~according to any one of aspect 1 and embodiments 2 to 30~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to FAP and comprise a variable heavy chain domain comprising an amino acid sequence of SEQ ID NO: 15 and a variable light chain domain comprising an amino acid sequence of SEQ ID NO: 16, or a variable heavy chain domain comprising an amino acid sequence of

SEQ ID NO: 66 and a variable light chain domain comprising an amino acid sequence of SEQ ID NO: 67, or wherein the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 97 and the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 98.

32. The method ~~as described above according to any one of aspect 1 and embodiments 2 to 31~~, wherein (i) the antibody heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 15 and the antibody light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 16, or the antibody heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 66 and the antibody light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 67, (ii) the first fusion polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 68, SEQ ID NO: 71 and SEQ ID NO: 73, and the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 14, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 72 and SEQ ID NO: 74.
33. The method ~~as described above according to any one of aspect 1 and embodiments 2 to 32~~, wherein (i) the antibody heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 15 and the antibody light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 16, or the antibody heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 66 and the antibody light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 67, (ii) the first fusion polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79 and SEQ ID NO: 82, and the second fusion polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80 and SEQ ID NO: 83.
34. The method ~~as described above according to any one of claims 1 to 28 and 42 to 47~~, wherein the antibody heavy chain and the antibody light chain form a binding site specifically binding to (human) FAP and the antibody heavy chain has the amino acid sequence of SEQ ID NO: 141 and the light chain has the amino acid

sequence of SEQ ID NO: 142, wherein the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 79, and the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 80.

35. The method ~~as described above according to any one of aspect 1 or embodiments 2 to 28~~, wherein the antigen is CEA.
36. The method ~~as described above according to any one of aspect 1 and embodiments 2 to 28 and 35~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to CEA and comprise a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 84, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 85, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 86, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 87, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 88, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 89.
37. The method ~~as described above according to any one aspect 1 and embodiments 2 to 28 and 35 to 36~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to CEA and comprise a variable heavy chain domain comprising an amino acid sequence of SEQ ID NO: 90 and a variable light chain domain comprising an amino acid sequence of SEQ ID NO: 91.
38. The method ~~as described above according to any one of aspect 1 and embodiments 2 to 28 and 35 to 37~~, wherein antibody-multimer-fusion comprises
 - (i) a heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO: 90 and a light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO: 91,
 - (ii) a first fusion polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 68, SEQ ID NO: 71 and SEQ ID NO: 73, and

(iii) a second fusion polypeptide comprising the amino acid sequence of SEQ ID NO: 14, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 72 and SEQ ID NO: 74.

39. The method ~~as described above according to any one of aspect 1 and embodiments 2 to 28 and 35 to 38~~, wherein antibody-multimer-fusion comprises

(i) a heavy chain comprising the VH domain comprising the amino acid sequence of SEQ ID NO: 90 and a light chain comprising the VL domain comprising the amino acid sequence of SEQ ID NO: 91,

(ii) a first fusion polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79 and SEQ ID NO: 82, and

(iii) a second fusion polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80 and SEQ ID NO: 83.

40. The method ~~as described above according to any one aspect 1 and embodiments 2 to 28 and 35 to 39~~, wherein antibody-multimer-fusion comprises

(i) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 93, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 94, and two light chains comprising the amino acid sequence of SEQ ID NO: 92, or

(ii) a first heavy chain comprising the amino acid sequence of SEQ ID NO: 95, a second heavy chain comprising the amino acid sequence of SEQ ID NO: 96, and two light chains comprising the amino acid sequence of SEQ ID NO: 92.

41. The method ~~as described above according to any one of aspect 1 or embodiments 2 to 28 and 35 to 40~~, wherein the antibody heavy chain and the antibody light chain form a binding site specifically binding to (human) CEA and the antibody heavy chain

has the amino acid sequence of SEQ ID NO: 143 and the light chain has the amino acid sequence of SEQ ID NO: 92, wherein the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 79, and the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 80.

42. The method as described above~~according to any one of aspects 1 or embodiments 2 to 28~~, wherein the antigen is CD19.
43. The method as described above~~according to any one aspect 1 or embodiments 2 to 28 and 42~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to CD19 and comprise a VH domain comprising (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 104 or SEQ ID NO: 105, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 106 or SEQ ID NO: 107, and (iii) CDR-H3 comprising the amino acid sequence of SEQ ID NO: 108 or SEQ ID NO: 109, and a VL domain comprising (iv) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 110 or SEQ ID NO: 111, (v) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 112 or SEQ ID NO: 113, and (vi) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 114 or SEQ ID NO: 115.
44. The method as described above~~according to any one aspect 1 or embodiments 2 to 28 and 42 to 43~~, wherein the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to CD19 and comprise a variable heavy chain domain comprising an amino acid sequence of SEQ ID NO: 116 and a variable light chain domain comprising an amino acid sequence of SEQ ID NO: 117, or comprise a variable heavy chain domain comprising an amino acid sequence of SEQ ID NO: 118 and a variable light chain domain comprising an amino acid sequence of SEQ ID NO: 119.
45. The method as described above~~according to any one aspect 1 or embodiments 2 to 28 and 42 to 44~~, wherein
 - (i) the heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 116 and the light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 117, or

the heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 118 and the light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 119,

(ii) the first fusion polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 68, SEQ ID NO: 71 and SEQ ID NO: 73, and

(iii) the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 14, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 72 and SEQ ID NO: 74.

46. The method as described above~~according to any one aspect 1 or embodiments 2 to 28 and 42 to 45~~, wherein

(i) the heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 116 and the light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 117, or the heavy chain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 118 and the light chain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 119,

(ii) the first fusion polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79 and SEQ ID NO: 82, and

(iii) the second fusion polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80 and SEQ ID NO: 83.

47. The method as described above~~according to any one of aspect 1 or embodiments 2 to 28 and 42 to 45~~, wherein

(i) the heavy chain comprises the amino acid sequence of SEQ ID NO: 120, the first fusion polypeptide comprises the amino acid sequence of SEQ ID

NO: 121, and the light chain comprises the amino acid sequence of SEQ ID NO: 122, or

(ii) the heavy chain comprises the amino acid sequence of SEQ ID NO: 123, the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 124, and the light chain comprises the amino acid sequence of SEQ ID NO: 122, or

(iii) the heavy chain comprises the amino acid sequence of SEQ ID NO: 125, the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 126, and the light chain comprises the amino acid sequence of SEQ ID NO: 127, or

(iv) the heavy chain comprises the amino acid sequence of SEQ ID NO: 128, the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 129, and the light chain comprises the amino acid sequence of SEQ ID NO: 127.

48. The method ~~as described above according to any one of aspect 1 or embodiments 2 to 28 and 42 to 47~~, wherein the antibody heavy chain and the antibody light chain form a binding site specifically binding to (human) CD19 and the antibody heavy chain has the amino acid sequence of SEQ ID NO: 144 and the light chain has the amino acid sequence of SEQ ID NO: 127, wherein the first fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 79, and the second fusion polypeptide comprises the amino acid sequence of SEQ ID NO: 80.
49. The method ~~as described above according to any one of aspect 1 or embodiments 2 to 48~~, wherein the transfecting a (parent) mammalian cell is a targeted integration transfecting.
50. The method ~~as described above according to embodiment 49~~, wherein the targeted integration transfecting is a double recombinase mediated cassette exchange.
51. The method ~~as described above according to any one of embodiments 49 to 50~~, wherein the (parent) mammalian cell is a CHO cell harboring a landing site integrated at a single site within a locus of its genome.

52. The method as described above~~according to embodiment 51~~, wherein the landing site comprises a first and a second selection marker, which are flanked by two RRSs, wherein the first selection marker is different from the second selection marker.
53. The method as described above~~according to embodiment 52~~, wherein the first selection marker is a glutamine synthetase selection marker and the second selection marker is a GFP fluorescent protein.
54. The method as described above~~according to embodiment 52~~, wherein the integrated landing site comprises a thymidine kinase selection marker and a HYG selection marker.
55. The method as described above~~according to any one of embodiments 52 to 54~~, wherein the two RRSs flanking both selection markers are different.
56. The method as described above~~according to any one of embodiments 51 to 55~~, wherein the landing site comprises three heterospecific loxP sites for Cre recombinase mediated DNA recombination.
57. The method as described above~~according to embodiment 56~~, wherein the heterospecific loxP sites are L3, LoxFas and 2L.
58. The method as described above~~according to embodiment 57~~, wherein the L3 and 2L flank the landing site at the 5'-end and 3'-end, respectively, and LoxFas is located between the L3 and 2L sites.
59. The method as described above~~according to any one of embodiments 51 to 58~~, wherein the landing site further contains a bicistronic unit linking the expression of a selection marker via an IRES to the expression of the fluorescent GFP protein.
60. The method as described above~~according to any one of aspect 1 or embodiments 2 to 59~~, wherein the antibody-multimer-fusion polypeptide is expressed from a deoxyribonucleic acid integrated in the genome of the cell that comprises in 5'- to 3'-direction
 - a first expression cassette encoding the first fusion polypeptide,
 - a second expression cassette encoding the first fusion polypeptide,
 - a third expression cassette encoding the second fusion polypeptide,

- a fourth expression cassette encoding the antibody heavy chain, and
 - a fifth expression cassette encoding the antibody light chain.
61. The method as described above~~according to any one of aspect 1 or embodiments 2 to 60~~, wherein the deoxyribonucleic acid encoding the antibody-multimer-fusion polypeptide is stably integrated into the genome of the mammalian cell at a single site or locus.
62. The method as described above~~according to any one of embodiments 60 to 61~~, wherein the deoxyribonucleic acid encoding the antibody-multimer-fusion polypeptide further comprises
- a first recombination recognition sequence located 5' to the first (most 5') expression cassette,
 - a second recombination recognition sequence located 3' to the fifth (most 3') expression cassette, and
 - a third recombination recognition sequence located
 - between the first and the second recombination recognition sequence, and
 - between the third and the fourth expression cassette,
- and
- wherein all recombination recognition sequences are different.
63. The method as described above~~according to any one of embodiments 60 to 62~~, wherein the deoxyribonucleic acid encoding the antibody-multimer-fusion polypeptide further comprises a further expression cassette encoding for a selection marker.
64. The method as described above~~according to embodiment 63~~, wherein the expression cassette encoding for a selection marker is located either
- i) 5', or
 - ii) 3', or
 - iii) partly 5' and partly 3'
- to the third recombination recognition sequence.
65. The method as described above~~according to any one of embodiments 63 to 64~~, wherein the expression cassette encoding for a selection marker is located partly 5' and partly 3' to the third recombination recognition sequences, wherein the 5'-located part of said expression cassette comprises the promoter and a start-codon

and the 3'-located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal.

66. The method as described above~~according to any one of embodiments 60 to 65~~, wherein the deoxyribonucleic acid encoding the antibody-multimer-fusion polypeptide comprises a further expression cassette encoding for a selection marker and the expression cassette encoding for the selection marker is located partly 5' and partly 3' to the third recombination recognition sequence, wherein the 5'-located part of said expression cassette comprises the promoter and the start-codon and the 3'-located part of said expression cassette comprises the coding sequence without a start-codon and a polyA signal, wherein the start-codon is operably linked to the coding sequence.
67. The method as described above~~according to any one of embodiments 65 to 66~~, wherein the start-codon is ATG.

Detailed Description of Embodiments of the Invention

The current invention is based, at least in part, on the finding that for the expression of an antibody-multimer-fusion, which is a complex molecule comprising different polypeptides, i.e. which is a heteromultimer, the use of a defined expression cassette ratio results in efficient expression and production of the antibody-multimer-fusion in mammalian cells, such as HEK or CHO cells.

The current invention is based, at least in part, on the finding that for transient as well as stable expression of an antibody-multimer-fusion, which is a complex molecule comprising different polypeptides, i.e. which is a heteromultimer, the use of the same defined expression cassette ratio results in the highest expression yield and product quality.

I. DEFINITIONS

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and is referred to as "numbering according to Kabat" herein. Specifically, the Kabat numbering system (see pages 647-660) of Kabat, et al., Sequences of Proteins of