

Patent claims

1. 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 claim 1, wherein the antibody-multimer-fusion polypeptide is transiently or stably expressed.

3. The method according to any one of claim 1 or claim 2, wherein the mammalian cell is a CHO cell.
4. The method according to any one of claims 1 to 3, 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 claims 1 to 3, 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 claim 5, 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 claims 1 to 6, 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 claim 7, 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.
9. The method according to claim 7, 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.

10. The method according to any one of claims 1 to 9, 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.
11. The method according to any one of claims 7 to 10, wherein the TNF ligand family member is 4-1-BBL.
12. The method according to any one of claims 7 to 11, wherein the ectodomain of a TNF ligand family member comprises the amino acid sequence of SEQ ID NO: 01 or SEQ ID NO: 56.
13. The method according to any one of claims 1 to 12, 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.
14. The method according to any one of claims 1 to 13, wherein the antigen is Fibroblast Activation Protein (FAP).
15. The method according to any one of claims 1 to 13, wherein the antigen is CEA.
16. The method according to any one of claims 1 to 13, wherein the antigen is CD19.