Patent Claims

1. A method for the recombinant production of a complex comprising

i) a fusion polypeptide ~~of~~ comprising in N- to C-terminal direction a T-cell response eliciting peptide, β2-microglobulin and the extracellular domains α1, α2, and α3 of a class I MHC molecule,

ii) a pair of disulfide-linked polypeptide chains derived from an antibody hinge region, wherein each disulfide-linked polypeptide chain comprises a CH2 domain and a CH3 domain of human origin, and

iii) at least one pair of an antibody light chain variable domain and an antibody heavy chain variable domain in a eukaryotic cell, comprising the following steps:

- cultivating a eukaryotic cell comprising one or more nucleic acids encoding the complex, and
- recovering the complex from the cell or the cultivation medium,

wherein the complex comprises exactly one fusion polypeptide of β2-microglobulin and the extracellular domains α1, α2, and α3 of a class I MHC molecule.

2. The method according to claim 1, characterized in that the complex is obtained with a concentration of 1 mg/ml or more in the cultivation medium.

3. The method according to any one of the preceding claims, characterized in that the eukaryotic cell is a mammalian cell.

4. The method according to claim 3, characterized in that the mammalian cell is a human embryonic kidney cell, or a chinese hamster ovary cell, or a baby hamster kidney cell, or a mouse myeloma cell.

~~5. The method according to any one of the preceding claims, characterized in that the fusion polypeptide comprises in N- to C-terminal direction a β2-microglobulin, and the extracellular domains α1, α2, and α3 of a class I MHC molecule with a relative frequency of less than 1 %.~~

~~6. The method according to any one of claims 1 to 4, characterized in that the fusion polypeptide comprises a T-cell response eliciting peptide, a β2-microglobulin, and the extracellular domains α1, α2, and α3 of a class I MHC molecule with a relative frequency of 1 % or more.~~

~~7~~5. The method according to any one of the preceding claims, characterized in that the disulfide-linked polypeptide chains derived from an antibody hinge region are i) linked by one or more disulfide bonds, ii) the first disulfide-

linked polypeptide chain comprises in N- to C-terminal direction an immunoglobulin light or heavy chain variable domain, an immunoglobulin light or heavy chain constant domain, and an antibody heavy chain hinge region polypeptide, and the second disulfide-linked polypeptide chain comprises an antibody heavy chain hinge region polypeptide.

~~8~~6. The method according to any one of the preceding claims, characterized in that the fusion polypeptide is i) covalently bound either to the C-terminus or the N-terminus of one of the disulfide-linked polypeptide chains, or ii) covalently bound to the N-terminus of an antibody variable domain that is the complementary cognate heavy or light chain variable domain to that comprised in the first disulfide-linked polypeptide chain, or iii) covalently bound to the C-terminus of an antibody constant domain that is the complementary heavy or light chain constant domain to that comprised in the first disulfide-linked polypeptide chain.

~~9~~7. The method according to any one of the preceding claims ~~6 to 8~~, characterized in that the T-cell response eliciting peptide is a virus-derived peptide.

~~10~~8. The method according to any one of the preceding claims ~~6 to 9~~, characterized in that the fusion polypeptide comprises in N- to C-terminal direction

(i) a virus-derived peptide that has an amino acid sequence selected from SEQ ID NO: 01 to SEQ ID NO: 09,

(ii) a first linker peptide that has an amino acid sequence selected from SEQ ID NO: 16, 17, 18, 21, 22, and 23,

(iii) a β2-microglobulin that has an amino acid sequence of SEQ ID NO: 10,

(iv) a second linker peptide that has an amino acid sequence selected from SEQ ID NO: 16, 17, 18, 21, 22, and 23,

(v) the extracellular domains α1, α2, and α3 of a class I MHC molecule that has an amino acid sequence of SEQ ID NO: 11, and

(vi) a third linker peptide that has an amino acid sequence selected from SEQ ID NO: 12, 16, 17, 18, 21, 22, and 23.

~~11~~9. The method according to any one of the preceding claims, characterized in that the first disulfide-linked polypeptide chain and the second disulfide-linked polypeptide chain comprise i) a human IgG1 CH2 domain comprising an amino acid sequence selected from SEQ ID NO: 31, 32, and 33, and a

human IgG1 CH3 domain comprising an amino acid sequence selected from SEQ ID NO: 34, 35, and 36.

~~12~~10. The method according to any one of claims ~~10~~ 8 to ~~11~~9, characterized in that the complex comprises i) a first linker peptide that has the amino acid sequence of SEQ ID NO: 21, and/or ii) a second linker peptide that has the amino acid sequence of SEQ ID NO: 22, and/or iii) a third linker peptide that has the amino acid sequence of SEQ ID NO: 12, and/or iv) a human IgG1 CH2 domain that has the amino acid sequence of SEQ ID NO: 32 or 33, and/or v) in the first disulfide-linked polypeptide a human IgG1 CH3 domain that has the amino acid sequence of SEQ ID NO: 35 and in the second disulfide-linked polypeptide a human IgG1 CH3 domain that has the amino acid sequence of SEQ ID NO: 36.

~~13~~11. A complex, characterized in that it comprises

- one fusion polypeptide that comprises in N- to C-terminal direction

~~either~~

~~(i) a β2-microglobulin, and~~

~~(ii) the extracellular domains α1, α2, and α3 of a class I MHC molecule with a relative frequency of less than 1 %,~~

~~or~~

(i) a T-cell response eliciting peptide,

(ii) a β2-microglobulin, and

(iii) the extracellular domains α1, α2, and α3 of a class I MHC molecule ~~with a relative frequency of 1 % or more~~,

and

- two polypeptide chains, which are linked by one or more disulfide bonds, wherein the first disulfide-linked polypeptide chain comprises in N- to C-terminal direction

(i) an immunoglobulin light or heavy chain variable domain,

(ii) an immunoglobulin light or heavy chain constant domain, and

(iii) an antibody heavy chain hinge region polypeptide,

and

wherein the second disulfide-linked polypeptide chain comprises an antibody heavy chain hinge region polypeptide, and

wherein the first disulfide-linked polypeptide chain and the second disulfide-linked polypeptide chain comprise, respectively, a CH2 domain and a CH3 domain of human origin,

wherein the fusion polypeptide is

- covalently bound either to the C-terminus or the N-terminus of one of the disulfide-linked polypeptide chains, or
- covalently bound to the N-terminus of an antibody variable domain that is the complementary heavy or light chain variable domain to that comprised in the first disulfide-linked polypeptide chain, or
- covalently bound to the C-terminus of an antibody constant domain that is the complementary heavy or light chain constant domain to that comprised in the first disulfide-linked polypeptide chain

with the proviso that the complex comprises exactly one fusion polypeptide.

~~14~~12. The complex of claim ~~13~~11, characterized in that the T-cell response eliciting peptide is a virus-derived peptide.

~~15~~13. The complex of any one of claims ~~13–~~11 or ~~14~~12, characterized in that the fusion polypeptide comprises in N- to C-terminal direction

(i) a virus-derived peptide that has an amino acid sequence selected from SEQ ID NO: 01 to SEQ ID NO: 09,

(ii) a first linker peptide that has an amino acid sequence selected from SEQ ID NO: 16, 17, 18, 21, 22, and 23.

(iii) a β2-microglobulin that has an amino acid sequence of SEQ ID NO: 10,

(iv) a second linker peptide that has an amino acid sequence selected from SEQ ID NO: 16, 17, 18, 21, 22, and 23.

(v) the extracellular domains α1, α2, and α3 of a class I MHC molecule that has an amino acid sequence of SEQ ID NO: 11, and

(vi) a third linker peptide that has an amino acid sequence selected from SEQ ID NO: 12, 16, 17, 18, 21, 22, and 23.

~~16~~14. The complex according to any one of claims ~~13–~~11 to ~~15~~13, characterized in that the first disulfide-linked polypeptide and the second disulfide-linked polypeptide further comprise

- a human IgG1 CH2 domain comprising an amino acid sequence selected from SEQ ID NO: 31, 32, and 33, and
- a human IgG1 CH3 domain comprising an amino acid sequence selected from SEQ ID NO: 34, 35, and 36.

~~17~~15. The complex according to any one of claims ~~15~~-13 to ~~16~~14, characterized in that

- the first linker peptide has the amino acid sequence of SEQ ID NO: 21, and/or
- the second linker peptide has the amino acid sequence of SEQ ID NO: 22, and/or
- the third linker peptide has the amino acid sequence of SEQ ID NO: 12, and/or
- the human IgG1 CH2 domain has the amino acid sequence of SEQ ID NO: 32 or 33, and/or
- the human IgG1 CH3 domain of one disulfide-linked polypeptide has the amino acid sequence of SEQ ID NO: 35 and the human IgG1 CH3 domain of the other disulfide-linked polypeptide has the amino acid sequence of SEQ ID NO: 36.

~~18~~16. A nucleic acid encoding the complex of any one of claims ~~13~~-11 to ~~17~~15.

~~19~~17. A host cell comprising the nucleic acid of claim ~~18~~16.

~~20~~18. A pharmaceutical formulation comprising the complex according to any one of claims ~~13~~ 11 to ~~17~~-15 and optionally a pharmaceutically acceptable carrier.

~~21~~19. The complex according to any one of claims ~~13~~-11 to ~~17~~-15 for use as a medicament.

~~22~~20. The complex according to any one of claims ~~13~~-11 to ~~17~~-15 for use in treating cancer or a chronic viral infection.

~~23~~21. The complex according to any one of claims ~~13~~–11 to ~~17~~–15 for use in attracting virus-specific cytotoxic T-cells of an individual to a target.

~~24~~22. The complex according to any one of claims ~~13~~-11 to ~~17~~-15 for use in removal of cancer cells or virus infected cells.

~~25~~23. Use of the complex according to any one of claims ~~13~~-11 to ~~17~~-15 in the manufacture of a medicament.

~~26~~24. The use of claim ~~25~~23, wherein the medicament is for treatment of cancer or a chronic viral infection.

~~27~~25. The use of claim ~~25~~23, wherein the medicament is for attracting virus-specific cytotoxic T-cells of an individual to a target.

~~28~~26. The use of claim ~~25~~23, wherein the medicament is for removal cancer cells or virus infected cells.

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