Claims

1. An immunoconjugate comprising a first and a second ~~antigen binding moiety~~Fab molecule, an Fc domain consisting of two subunits, and ~~an effector moiety~~a cytokine, wherein said first and said second Fab molecule and said Fc domain are part of an immunoglobulin molecule and wherein not more than one ~~effector moiety~~cytokine is present.

~~2. The immunoconjugate of claim 1, wherein said effector moiety is fused to the amino- or carboxy-terminal amino acid of one of said two subunits of the Fc domain, optionally through a linker peptide.~~

~~3. The immunoconjugate of claim 1, wherein said first antigen binding moiety is fused to the amino-terminal amino acid of one of said two subunits of the Fc domain, optionally through a linker peptide or an immunoglobulin hinge region.~~

~~4. The immunoconjugate of any one of claims 1 to 3, wherein said first antigen binding moiety comprises an antigen binding domain of an antibody.~~

~~5. The immunoconjugate of claim 4, wherein said first antigen binding moiety is a Fab molecule.~~

~~6~~2. The immunoconjugate of ~~any one of~~ claims ~~1 to 5~~, wherein said Fc domain comprises a modification promoting heterodimerization of two non-identical polypeptide chains.

~~7~~3. The immunoconjugate of claim ~~6~~2, wherein said modification is a knob-into-hole modification, comprising a knob modification in one of the subunits of the Fc domain and a hole modification in the other one of the two subunits of the Fc domain, wherein the knob modification comprises a protuberance constructed by replacing an amino acid with a small side chain with an amino acid with a larger side chain, and the hole modification comprises a cavity of identical or similar size to the protuberance constructed by replacing an amino acid with a large side chain with an amino acid with a smaller side chain.

~~8~~4. The immunoconjugate of claim ~~7~~3, wherein said effector moiety is fused to the amino- or carboxy-terminal amino acid of the subunit of the Fc domain comprising the knob modification.

~~9. The immunoconjugate of any one of claims 1 to 8, wherein said Fc domain is an IgG Fc domain.~~

~~10. The immunoconjugate of any one of claims 1 to 9, wherein said Fc domain is engineered to have altered binding to an Fc receptor and/or altered effector function.~~

~~11. The immunoconjugate of claim 10, wherein said Fc receptor is an Fcγ receptor.~~

~~12. The immunoconjugate of claim 10, wherein said effector function is ADCC.~~

~~13. The immunoconjugate of claim 10, wherein said altered binding and/or effector function is reduced binding and/or effector function.~~

~~14~~5. The immunoconjugate of ~~claim 13~~any one of claims 1 to 4, wherein said Fc domain comprises one or more amino acid mutation that reduces the binding of the Fc domain to an Fc receptor, particularly an Fcγ receptor.

~~15~~6. The immunoconjugate of claim ~~14~~5, wherein said amino acid mutation is an amino acid substitution at position P329 (EU numbering).

~~16~~7. The immunoconjugate of claims ~~14~~ 5 or ~~15~~6, wherein the Fc domain comprises the amino acid substitutions L234A, L235A and P329G (EU numbering) in each of its subunits.

~~17. The immunoconjugate of claim 10, wherein said altered binding and/or effector function is increased binding and/or effector function.~~

~~18. The immunoconjugate of claim 17, wherein said Fc domain is engineered to have an altered oligosaccharide structure, as compared to a non-engineered Fc domain.~~

~~19. The immunoconjugate of claim 18, wherein said Fc domain comprises an increased proportion of non-fucosylated oligosaccharides, as compared to a non-engineered Fc domain.~~

~~20. The immunoconjugate of any of claims 1 to 19, wherein the immunoconjugate comprises a first and a second antigen binding moiety.~~

~~21. The immunoconjugate of claim 20, wherein said first and said second antigen binding moiety and said Fc domain are part of an immunoglobulin molecule.~~

~~22~~8. The immunoconjugate of ~~claim 21~~any one of claims 1 to 7, wherein said immunoglobulin molecule is an IgG class immunoglobulin.

~~23~~9. The immunoconjugate of ~~claims 20 or 21~~any one of claims 1 to 8, wherein said ~~effector moiety~~cytokine is fused to the carboxy-terminal amino acid of one of the immunoglobulin heavy chains, optionally through a linker peptide.

~~24~~10. The immunoconjugate of any one of claims 1 to ~~23~~9, wherein ~~said first antigen binding moiety, or~~ said first and said second ~~antigen binding moiety,~~Fab molecule is directed to an antigen associated with a pathological condition.

~~25~~11. The immunoconjugate of any one of claims 1 to ~~24~~10, wherein ~~said first antigen binding moiety, or~~ said first and said second ~~antigen binding moiety,~~Fab molecule is directed to an antigen selected from the group of Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB),

Carcinoembryonic Antigen (CEA), and Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

~~26~~12. The immunoconjugate of any one of claims 1 to ~~25~~11, wherein ~~said first antigen binding moiety, or~~ said first and said second ~~antigen binding moiety,~~Fab molecule is directed to CEA.

~~27~~13. The immunoconjugate of claim ~~26~~12, wherein ~~said first antigen moiety, or~~ said first and said second ~~antigen binding moiety,~~Fab molecule comprises a heavy chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 191, and a light chain variable region sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO: 189.

~~28~~14. The immunoconjugate of any one of claims 1 to ~~27~~13, wherein said ~~effector moiety~~cytokine is a single chain ~~effector moiety~~cytokine.

~~29. The immunoconjugate of any one of claims 1 to 28, wherein the effector moiety is a cytokine.~~

~~30~~15. The immunoconjugate of any one of claims 1 to 14~~29~~, wherein said cytokine is IL-2.

~~31~~16. The immunoconjugate of any one of claims ~~29 or 30~~1 to 15, wherein said cytokine is a mutant IL-2 polypeptide having reduced binding affinity to the α-subunit of the IL-2 receptor.

~~32~~17. The immunoconjugate of claim ~~31~~16, wherein said mutant IL-2 polypeptide comprises an amino acid substitution at one or more positions selected from the positions corresponding to residues 42, 45 and 72 of human IL-2.

~~33~~18. The immunoconjugate of claim ~~32~~17, wherein said mutant IL-2 polypeptide comprises the amino acid substitutions F42A, Y45A and L72G.

~~34~~19. The immunoconjugate of any one of claims 1 to ~~33~~18, comprising the polypeptide sequences of SEQ ID NO: 277, SEQ ID NO: 281 and SEQ ID NO: 283, or variants thereof that retain functionality.

~~35. The immunoconjugate of claim 29, wherein said cytokine is IL-10.~~

~~36~~20. An isolated polynucleotide encoding the immunoconjugate of any one of claims 1 to ~~35~~19 or a fragment thereof.

~~37~~21. An expression vector comprising the isolated polynucleotide of claim ~~36~~20.

~~38~~22. A host cell comprising the isolated polynucleotide of claim ~~36~~ 20 or the expression vector of claim ~~37~~21.

~~39. The host cell of claim 38, wherein said host cell has been manipulated to express increased levels of one or more polypeptides having β(1,4) N-acetylglucosaminyltransferase III (GnTIII) activity.~~

~~40~~23. A method of producing ~~the~~ an immunoconjugate ~~of any one of claims 1 to 31~~comprising a first and a second antigen binding Fab molecule, an Fc domain, and a single cytokine, comprising culturing the host cell of claim ~~38 or 39~~22 under conditions suitable for the expression of the immunoconjugate and optionally recovering the immunoconjugate.

~~41~~24. An immunoconjugate comprising ~~an~~ a first and a second antigen binding ~~moiety~~Fab molecule, an Fc domain and a single ~~effector moiety~~cytokine, wherein said immunoconjugate is produced by the method of claim ~~40~~23.

~~42~~25. A pharmaceutical composition comprising the immunoconjugate of any one of claims 1 to ~~35~~ 19 or claim ~~41~~ 24 and a pharmaceutically acceptable carrier.

~~43~~26. The immunoconjugate of any one of claims 1 to ~~35~~ 19 or claim ~~41~~24, or the pharmaceutical composition of claim ~~42~~25, for use in the treatment of a disease in an individual in need thereof.

~~44~~27. The immunoconjugate of any one of claims 1 to ~~35~~ 19 or claim ~~41~~24, or the pharmaceutical composition of claim ~~42~~25, for the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

~~45~~28. The immunoconjugate or the pharmaceutical composition of claims ~~43~~ 26 or ~~44~~27, wherein said disease is cancer.

~~46. The immunoconjugate or the pharmaceutical composition of claims 43 or 44, wherein said disease is an inflammatory disorder.~~

~~47. The immunoconjugate or pharmaceutical composition of claim 46, wherein the effector moiety of the immunoconjugate is IL-10.~~