Claims

1. Antibody, or antigen binding fragment thereof, comprising a first polypeptide chain and a second polypeptide chain,

wherein said first polypeptide chain comprises in N- to C-terminal direction

(i) one or more variable domains;

(ii) a functional domain; and

(iii) one or more constant domains; ~~and~~

wherein said second polypeptide chain comprises in N- to C-terminal direction

(iv) one or more variable domains forming antigen binding sites with the one or more variable domains of the first polypeptide chain;

(v) (a) no functional domain ~~optionally,~~ or (b) a functional domain; and

(vi) one or more constant domains,

characterized in that said functional domain (ii) of the first polypeptide chain does not comprise a fragment of the second polypeptide chain and said optional functional domain (v) of the second polypeptide chain does not comprise a fragment of the first polypeptide chain,

wherein the functional domain (ii) of the first polypeptide chain comprises or consists of a carrier domain, a reporter domain, a tag, a localization domain, an (independent) binding site, an enzyme or enzymatic domain, a receptor or a functional fragment thereof, or a ligand or a functional fragment thereof.~~-~~

~~2. The antibody, or the antigen binding fragment thereof, according to claim 1, wherein the second polypeptide chain comprises no functional domain (v)~~ and wherein the C-terminus of the most C-terminal variable domain of the second polypeptide chain is directly coupled to the N-terminus of the most N-terminal constant domain of the second polypeptide chain.

~~3.~~2. The antibody, or the antigen binding fragment thereof, according to claim 1 ~~or 2~~, wherein

(a) the one or more constant domains of the first polypeptide chain is/are heavy chain constant domains comprising at least a CH1 constant domain, the one or more variable domains of the first polypeptide chain is/are heavy chain variable domains VH, the constant domain of the second polypeptide chain is a light chain constant domain CL and the one or more variable domains of the second polypeptide chain is/are light chain variable domains VL; or

(b) the constant domains of the first polypeptide chain is a light chain constant domain CL, the one or more variable domains of the first polypeptide chain is/are light chain variable

domains VL, the one or more constant domains of the second polypeptide chain is/are heavy chain constant domains comprising at least a CH1 constant domain and the one or more variable domains of the second polypeptide chain is/are heavy chain variable domains VH.

~~4.~~3. The antibody, or the antigen binding fragment thereof, according to claim ~~3~~2, wherein the CH1 domain, and optionally any further heavy chain constant domain, is selected from the following classes: γ, α, μ, ε and δ; preferably γ, such as from IgG1, IgG2, IgG3 or IgG4; and/or

wherein the CL domain is selected from the following classes: κ and λ.

~~5.~~4. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~4~~3, wherein the functional domain (ii) of the first polypeptide chain and/or the functional domain (v) of the second polypeptide chain comprises or consists of an Ig-like domain, preferably selected from the Ig-like domain(s) of PD1, SLAM, CD2, CD3, CD4, CD8, CD19, CD22, CD33, CD80, or CD86, more preferably CD4.

~~6.~~5. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~5~~4, wherein the functional domain (ii) of the first polypeptide chain and/or the functional domain (v) of the second polypeptide chain comprises or consists of:

- a carrier domain, preferably wherein the carrier domain provides for conjugation of the antibody, or the antigen binding fragment thereof, to a drug or to an imaging agent;
- a reporter domain, preferably wherein the reporter domain comprises or consists of an amino acid sequence coding for GFP, YFP, RFP, CFP, luciferase, beta-galactosidase or peroxidase;
- a tag, preferably wherein the tag is an affinity tag, a solubilization tag, a chromatography tag, an epitope tag or a fluorescence tag;
- a localization domain;
- an (independent) binding site;
- an enzyme or enzymatic domain, such as a catalytic domain;
- a receptor or a functional fragment thereof, for example an Ig-like domain of a receptor; or
- a ligand or a functional fragment thereof.

~~7.~~6. The antibody, or the antigen binding fragment thereof, according to claim ~~6~~5, wherein the functional domain comprises or consists of (i) a receptor selected from the group consisting of PD1, SLAM, LAIR1, CTLA4, BTLA, *TIM-3*, TIGIT, CD200R1, 2B4 (CD244), TLT2, LILRB4, KIR2DL2, ICOS or CD28, or (ii) a functional fragment of a receptor, such as an Ig-like domain of any one of the following receptors: PD1, SLAM, LAIR1, CTLA4, BTLA, *TIM-3*, TIGIT, CD200R1, 2B4 (CD244), TLT2, LILRB4, KIR2DL2, ICOS or CD28.

~~8.~~7. The antibody, or the antigen binding fragment thereof, according to claim ~~6~~5, wherein the functional domain (ii) of the first polypeptide chain and/or the functional domain (v) of the second polypeptide chain comprises or consists of a ligand or a functional fragment thereof, wherein

(1) the ligand is a cytokine and/or a ligand of PD1, SLAM, LAIR1, CTLA4, BTLA, *TIM-3*, TIGIT, CD200R1, 2B4 (CD244), TLT2, LILRB4, KIR2DL2, ICOS or CD28;

(2) the ligand is selected from the group consisting of PD-L1, PD-L2, B7-1, B7-2, B7-H4 (B7 homolog), galectin-9, poliovirus receptor (PVR), OX-2 membrane glycoprotein, CD48, B7-H3 (B7 homolog), MHCI, ICOS-L; or

(3) the ligand is selected from the group consisting of the SIS family of cytokines, the SIG family of cytokines, the SCY family of cytokines, the Platelet factor-4 superfamily and intercrines, CC chemokine ligands (CCL)-1 to -28, CXCL1 – CXCL17, XCL1 (lymphotactin-α) and XCL2 (lymphotactin-β), fractalkine (or CX_3CL1); interferons, such as Type I IFN, Type II IFN, and Type III IFN, in particular IFN-α, IFN-β, IFN-γ, IFN-ε, IFN-κ, IFN-ω, IL10R2 (also called CRF2-4) and IFNLR1 (also called CRF2-12); interleukins, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, and IL-36; lymphokines, such as IL-2, IL-3, IL-4, IL-5, IL-6, GM-CSF, and Interferon-gamma; tumor necrosis factors, such as CD40LG (TNFSF5); CD70 (TNFSF7); EDA; FASLG (TNFSF6); LTA (TNFSF1); LTB (TNFSF3); TNF, TNFα, TNFSF4 (OX40L); TNFSF8 (CD153); TNFSF9; TNFSF10 (TRAIL); TNFSF11 (RANKL); TNFSF12 (TWEAK); TNFSF13; TNFSF13B; TNFSF14; TNFSF15; and TNFSF18; and colony stimulating factors, such as CSF1 (macrophage colony-stimulating factor), CSF2 (GM-CSF), CSF3 (G-CSF), as well as synthetic CSFs, such as Promegapoietin.

~~9.~~8. The antibody, or the antigen binding fragment thereof, according to claim ~~6~~5, wherein the (independent) binding site comprises or consists of a receptor or a functional fragment thereof comprising a binding site, a ligand or a functional fragment thereof comprising a binding site, a CD molecule or a functional fragment thereof, a single chain antibody (such as scFv or VHH) or an antigen binding fragment thereof, an antigen or a functional fragment thereof, or a tag comprising a binding site.

~~10.~~9. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~9~~8, wherein the functional domain comprises or consists of an Ig-like domain, an scFv, a VHH or a Strep-tag, preferably wherein the functional domain comprises or consists of an amino acid sequence as set forth in any of SEQ ID NOs 13 – 20, or a functional sequence variant thereof having at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 98% sequence identity.

~~11.~~10. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~10~~9, wherein the first polypeptide chain comprises one single variable domain (i) N-terminal of the functional domain (ii) and the second polypeptide chain comprises one single variable domain (iv) N-terminal of the optional functional domain (v) or N-terminal of the one or more constant domains (vi), the one single variable domain (iv) of the second polypeptide chain forming an antigen binding site with the variable domain (i) of the first polypeptide chain; or

wherein the first polypeptide chain comprises two or more variable domains (i) N-terminal of the functional domain (ii) and the second polypeptide chain comprises two or more variable domains (iv) N-terminal of the optional functional domain (v) or N-terminal of the one or more constant domains (vi), the two or more variable domains (iv) of the second polypeptide chain forming antigen binding sites with the two or more variable domains (i) of the first polypeptide chain.

~~12.~~11. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~11~~10, wherein the first polypeptide chain comprises a single chain antibody, such as an scFv or a single domain antibody, for example a VHH, N-terminal of the most N-terminal variable domain (i) and/or C-terminal of the most C-terminal constant domain (iii); and/or

wherein the second polypeptide chain comprises a single chain antibody, such as an scFv or a single domain antibody, for example a VHH, N-terminal of the most N-terminal variable domain (i) and/or C-terminal of the most C-terminal constant domain (vi).

~~13.~~12. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~12~~11, wherein the first polypeptide chain and the second polypeptide chain each comprise one single constant domain, in particular a CL domain and a CH1 domain, respectively; or wherein the first polypeptide chain or the second polypeptide chain comprises one single constant domain, in particular a CL domain; and the other of the first polypeptide chain or the second polypeptide chain comprises a CH1 domain and one or more further constant domains, such as a CH2 and/or CH3 domain.

~~14.~~13. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~13~~12, wherein the first polypeptide chain comprises or consists of V-D-CH1, wherein

V is a variable domain (i);

D is a functional domain (ii); and

CH1 is a CH1 constant domain (iii); and/or

wherein the second polypeptide chain comprises or consists of V-CL, wherein

V is a variable domain (iv); and

CL is a constant domain (vi).

~~15.~~14. The antibody, or the antigen binding fragment thereof, according to claim ~~14~~13, wherein the first polypeptide chain comprises or consists of V-D-CH1-CH2-CH3, wherein CH2 and CH3 are a CH2 constant domain and a CH3 constant domain, respectively.

~~16.~~15. The antibody, or the antigen binding fragment thereof, according to claim ~~14~~ 13 or ~~15~~14, wherein the first polypeptide chain comprises or consists of $(V)_A$-D-CH1 with A being an integer from 1 to ~~5~~4, preferably from 1 to ~~4~~3, more preferably from 1 to ~~3~~ 2 and even more preferably 1 ~~or 2~~ and wherein the variable domains V may be coupled to each other directly or via a linker; and/or

wherein the second polypeptide chain comprises or consists of $(V)_A$-CL with A being an integer from 1 to ~~5~~4, preferably from 1 to ~~4~~3, more preferably from 1 to ~~3~~ 2 and even more preferably 1 ~~or 2~~ and wherein the variable domains V may be coupled to each other directly or via a linker.

~~17.~~16. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~16~~15, wherein the variable domain (i) of the first polypeptide chain comprises or consists of an amino acid sequence as set forth in any one of SEQ ID NOs 1, 5, 8 or 10, or a functional sequence variant thereof having at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 98% sequence identity; and/or

wherein the variable domain (iv) of the second polypeptide chain comprises or consists of an amino acid sequence as set forth in any one of SEQ ID NOs 2, 6, 9 or 11, or a functional sequence variant thereof having at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 98% sequence identity.

~~18.~~17. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~17~~16, wherein the antibody, or the antigen binding fragment thereof, comprises one single or two first polypeptide chains and one single or two second polypeptide chains.

~~19.~~18. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~18~~17, wherein the first polypeptide chain and the second polypeptide chain are linked by a disulfide bond, thereby forming a pair.

~~20.~~19. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~19~~18, wherein the antibody, or the antigen binding fragment thereof, is monovalent or bivalent for each specificity/antigen.

~~21.~~20. The antibody, or the antigen binding fragment thereof, according to claim ~~20~~19, wherein the antigen binding site(s) formed by the one or more variable domains of the first pair of first and second polypeptide chains and the antigen binding site(s) formed by the one or more variable domains of the second pair of first and second polypeptide chains are the same or distinct.

~~22.~~21. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~21~~20, wherein the antibody, or the antigen binding fragment thereof, comprises at least two functional domains, which may be the same or distinct.

~~23.~~22. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~22~~21, wherein the antibody, or the antigen binding fragment thereof, comprises two first polypeptide chains and two second polypeptide chains forming a first and a second pair of first and second polypeptide chains, and wherein the first pair of first and second polypeptide chains comprises at least one functional domain(s) and/or the second pair of first and second polypeptide chains comprises at least one functional domain(s).

~~24.~~23. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~23~~22, wherein the first polypeptide chain comprises or consists of an amino acid sequence as set forth in any one of SEQ ID NOs 53, 55, 56, 58, 59, 60, 61, 62, 64, 65, 66, 68, 69 or 70, or a functional sequence variant thereof having at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 98% sequence identity, and/or the second polypeptide chain comprises or consists of an amino acid sequence as set forth in any one of SEQ ID NOs 54, 57, 63, or 67, or a functional sequence variant thereof having at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and most preferably at least 98% sequence identity.

~~25. Nucleic acid molecule comprising a first polynucleotide encoding a first polypeptide chain of the antibody, or antigen-binding fragment thereof, according to any one of claims 1 – 24 and/or a second polypeptide encoding the second polypeptide chain of the antibody, or antigen-binding fragment thereof, according to any one of claims 1 – 24.~~

~~26.~~24. Nucleic acid molecule comprising a polynucleotide encoding the first polypeptide chain and the second polypeptide chain of the antibody, or antigen-binding fragment thereof, according to any one of claims 1 – ~~24~~23.

~~27.~~25. Vector comprising the nucleic acid molecule according to claim ~~25~~ 24~~or 26~~.

~~28.~~26. Host cell comprising the nucleic acid molecule according to claim ~~25~~ 24~~or 16~~, or the vector according to claim ~~27~~25.

~~29.~~27. Composition comprising the antibody, or antigen-binding fragment thereof, according to any one of claims 1 – ~~24~~23, the nucleic acid molecule according to claim ~~25~~ 24~~or 26~~, the vector according to claim ~~27~~ 25 or the host cell according to claim ~~28~~26.

~~30.~~28. A process ~~for~~ to ~~producing~~ produce the antibody, or antigen-binding fragment thereof, according to any one of claims 1 – ~~24~~ 23 comprising:

a) transforming an eukaryotic host cell by incorporating one or more nucleic acid molecule(s) according to claim ~~25 or 26~~ 24 encoding a first polypeptide chain and a second polypeptide chain;

b) cultivating the host cell under suitable conditions so that said nucleic acid molecules are expressed;

c) causing or allowing said first and second polypeptide chains to combine to form the antibody, or antigen-binding fragment thereof; and

d) optionally, purifying the antibody, or antigen-binding fragment thereof, from the culture medium.

~~31.~~29. The antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~24~~23, the nucleic acid molecule according to claim ~~25~~ 24~~or 26~~, the vector according to claim ~~27~~25, the host cell according to claim ~~28~~26, or the composition according to claim ~~29~~ 27 for use in medicine.

~~32.~~30. An assay for detecting an antigen or for quantification of antigen binding comprising:

a) incubating an antigen with the antibody, or the antigen binding fragment thereof, according to any one of claims 1 – ~~24~~ 23 under conditions which permit binding of the antigen to the polyvalent antibody; and

b) detecting antigen-antibody binding.