

EP 1 378 520 discloses a cyclic single strand trispecific antibody.

Summary

Herein is reported a single circular polypeptide as a novel target binder. In this circular polypeptide the N-terminal portion comprises a first part of a binding site and the C-terminal portion comprises the second part of the binding site. The first part of the binding site and the second part of the binding site associate with each other to form the complete or functional binding site. Thereby the polypeptide is circularized. The association can be non-covalently or covalently. In case the association is covalently it is not by a peptide bond, but, e.g. by a disulfide bond.

As reported herein is a (circular) (single chain) fusion polypeptide that (specifically) binds to a target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein

- the spacer domain is a polypeptide (forming a structural domain after folding),
- the first part of the binding domain is a polypeptide and is fused (either directly or) via a first (peptidic) linker to the N-terminus of the spacer domain,
- the second part of the binding domain is a polypeptide and is fused (either directly or) via a second (peptidic) linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain (of the same (single chain) fusion polypeptide) (associate/are associated with/covalently or non-covalently bind to each other and) form a (functional) binding site that specifically binds to the target.

One aspect as reported herein is a dimeric fusion polypeptide comprising a first fusion polypeptide specifically binding to a target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the binding domain is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain,

- the second part of the binding domain is a polypeptide that is fused either directly or via a second linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain of the same single chain fusion polypeptide associate and form a functional binding site that specifically binds to the target,

and a second fusion polypeptide specifically binding to a target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein

- the spacer domain is a polypeptide and comprises at least 25 amino acid residues,
- the first part of the binding domain is a polypeptide that is fused either directly or via a first linker to the N-terminus of the spacer domain,
- the second part of the binding domain is a polypeptide that is fused either directly or via a second linker to the C-terminus of the spacer domain,
- the first part of the binding domain and the second part of the binding domain of the same single chain fusion polypeptide associate and form a functional binding site that specifically binds to the target,

wherein the first and the second fusion polypeptide are identical or different and wherein the spacer domain of the first fusion polypeptide is covalently conjugated to the spacer domain of the second fusion polypeptide.

In one embodiment the (circular) (single chain) fusion polypeptide comprises exactly one part of the binding domain N-terminal to the spacer domain and exactly one, but different, part of the same binding domain C-terminal to the spacer domain.

In one embodiment the first part of the binding domain is an antibody heavy chain variable domain and the second part of the binding domain is an antibody light chain variable domain or vice versa.

In one embodiment the binding domain is a DutaFab, wherein one part of the binding domain comprises an antibody heavy chain variable domain (VH) and at least an N-terminal fragment of a (or a complete) first antibody heavy chain constant domain (CH1) and the respective other binding domain comprises an antibody light chain variable domain (VL) and at least an N-terminal fragment of a (or a complete) antibody light chain constant domain (CL), wherein the binding domain comprises two non-overlapping paratopes in the complementary pair of a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein the first paratope comprises residues from CDR1 and CDR3 of the VL domain and CDR2 of the VH domain, and the second paratope comprises residues from CDR1 and CDR3 of the VH domain and CDR2 of the VL domain.

As reported herein is a (dicircular) fusion polypeptide comprising a first (circular) fusion polypeptide as reported herein and a second (circular) fusion polypeptide as reported herein, wherein the first and the second (circular) fusion polypeptides are identical or different and wherein the spacer domain of the first (circular) fusion polypeptide is (covalently) conjugated to the spacer domain of the second (circular) fusion polypeptide.

In one embodiment the (dicircular) fusion polypeptide comprises

- a first (circular) (single chain) fusion polypeptide that (specifically) binds to a first target comprising a first part of a binding domain, a second part of a binding domain and a spacer domain, wherein
 - the spacer domain has an amino acid sequence of SEQ ID NO: 32 or SEQ ID NO: 38 or SEQ ID NO: 40,
 - the first part of the binding domain is an antibody heavy chain Fab fragment and the second part of the binding domain is an antibody light chain Fab fragment or vice versa,
 - the first part of the binding domain is fused via a first peptidic linker of SEQ ID NO: 64 or SEQ ID NO: 65 to the N-terminus of the spacer domain and the second part of the binding domain is fused via a second peptidic linker of SEQ ID NO: 64 or SEQ ID NO: 65 to the C-terminus of the spacer domain, whereby the first and the second peptidic linker are selected independently of each other,

In one embodiment in the constant domain CH1 of the second (circular) fusion polypeptide (of the multicircular fusion polypeptide) the amino acids at the positions corresponding to positions 147 and 213 according to the Kabat EU index are substituted by E.

5 In one embodiment one or each of the binding domain is a DutaFab, wherein one part of the binding domain comprises an antibody heavy chain variable domain (VH) and at least an N-terminal fragment of a (or a complete) first antibody heavy chain constant domain (CH1) and the respective other binding domain comprises an antibody light chain variable domain (VL) and at least an N-terminal fragment
10 of a (or a complete) antibody light chain constant domain (CL), wherein the binding domain comprises two non-overlapping paratopes in the complementary pair of a heavy chain variable domain (VH) and a light chain variable domain (VL), wherein the first paratope comprises residues from CDR1 and CDR3 of the VL domain and CDR2 of the VH domain, and the second paratope comprises residues
15 from CDR1 and CDR3 of the VH domain and CDR2 of the VL domain. One aspect as reported herein is an isolated nucleic acid encoding the circular fusion polypeptide as reported herein.

One aspect as reported herein is a pair of isolated nucleic acids together encoding the dimeric (circular) fusion polypeptide as reported herein.

20 One aspect as reported herein is a host cell comprising the nucleic acid as reported herein or the pair of nucleic acids as reported herein.

One aspect as reported herein is a method of producing a (circular or dicircular) fusion polypeptide comprising culturing the host cell as reported herein so that the (circular or dicircular) fusion polypeptide is produced and recovering the (circular or dicircular) fusion polypeptide from the cell or the cultivation medium.
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As reported herein is an immunoconjugate comprising the (circular) fusion polypeptide as reported herein and a cytotoxic agent.

One aspect as reported herein is a pharmaceutical formulation comprising the (circular) fusion polypeptide as reported herein or the (dimeric) (circular) fusion polypeptide as reported herein and a pharmaceutically acceptable carrier.
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