

binding domains in the final format. Major challenges with such formats have been (a) the relatively poor efficiency of specific hetero-dimerization resulting in suboptimal production yields, and (b) the necessity to use either non target binding proteins as hetero-dimerization domains or engineered hetero-dimer Fc effector domains that come with poor flexibility in tuning serum half-life and that limit the flexibility in adding novel functionalities without increasing the molecular weight.

Thus, the optimal hetero-dimeric multi-specific format would exclusively consist of target binding domains and would allow for adjusting the geometry of the molecule for example by freely changing the linker lengths between the different binding domains to accommodate the geometrical constraints defined by the interaction partners (targets). The solution to this problem, i.e. the modification of the order of the variable domains on the monomeric chains, has hitherto neither been shown nor suggested in the prior art.

SUMMARY

This disclosure relates to novel hetero-dimeric multi-specific format of multiple antibody variable domains comprising a core of two split variable domain pairs wherein both variable light domains and two cognate variable heavy domains are positioned in tandem on two separate protein chains, respectively, thereby driving hetero-dimerization of the two protein chains. Up to two additional binding domains, particularly antibody-based binding domains, such as scFv fragments, are fused to the amino- or the carboxyl-terminus of either protein chain, resulting in an up to hexa-specific hetero-dimeric protein.

Thus, in one aspect the present disclosure refers to a hetero-dimeric protein comprising a first and a second single-chain protein, wherein said first single-chain protein comprises a first amino acid sequence consisting of (from the N- to the C-terminus):

- (ia) a first VL domain;
- (iia) a first polypeptide linker,
- (iiia) a second VL domain,
- (iva) optionally, a first scFv fragment that is fused via a third polypeptide linker to said first VL domain, and
- (va) optionally, a second scFv fragment that is fused via a fourth polypeptide linker to said second VL domain, and

wherein said second single-chain protein comprises a second amino acid sequence consisting of (from the N- to the C-terminus):

- (ib) a first VH domain;
- (iib) a second polypeptide linker,
- (iiib) a second VH domain,
- (ivb) optionally, a third scFv fragment that is fused via a fifth polypeptide linker to said first VH domain, and
- (vb) optionally, a fourth scFv fragment that is fused via a sixth polypeptide linker to said second VH domain, and

provided that at least one of said first, second, third and fourth scFv fragments is present, wherein at least one of said VL domains comprises (i) human V κ framework regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3; and (iii) a framework region IV, which is a human V λ -based sequence for framework region IV.

In another aspect, the present disclosure refers to one or two nucleic acid sequences encoding said first and a second single-chain proteins. In a another aspect, the present disclosure refers to a nucleic acid sequence or two nucleic acid sequences encoding the first and the second single-chain proteins of the hetero-dimeric protein disclosed herein.

In yet another aspect, the present disclosure refers to one or two vectors comprising said one or two nucleic acid sequences. In a yet another aspect, the present disclosure refers to a vector or two vectors comprising the nucleic acid sequence or the two nucleic acid sequences disclosed herein.

In a further aspect, the present disclosure refers to a host cell or host cells comprising one or two vectors. In a yet another aspect, the present disclosure refers to a host cell or host cells comprising the vector or the two vectors disclosed herein.

In one aspect, the present disclosure refers to a method for producing the first and second single-chain proteins, or the hetero-dimeric protein, of the present disclosure, comprising (i) providing a nucleic acid or nucleic acids according to the present disclosure, or a vector or vectors according to the present disclosure, expressing said nucleic acid or nucleic acids or said vector or vectors and collecting said first and second single-chain proteins, or said hetero-dimeric protein, from the expression system, or (ii) providing a host cell or host cells of the present disclosure, culturing said host cell or host cells, and collecting said first and second

single-chain proteins, or said hetero-dimeric protein, from the cell culture. In yet another aspect, the present disclosure refers to a method for producing the hetero-dimeric protein disclosed herein, or the first and the second single-chain proteins of said hetero-dimeric protein, comprising (i) providing the nucleic acid sequence or the two nucleic acid sequences disclosed herein, or the vector or the two vectors disclosed herein, expressing said nucleic acid sequence or nucleic acid sequences, or said vector or vectors, and collecting said hetero-dimeric protein from the expression system, or (ii) providing a host cell or host cells disclosed herein, culturing said host cell or said host cells; and collecting said first and second single-chain proteins, or said hetero-dimeric protein, from the cell culture.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising the hetero-dimeric protein of the present disclosure and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the hetero-dimeric protein disclosed herein and a pharmaceutically acceptable carrier.

In yet another aspect, the present disclosure refers to the hetero-dimeric protein of the present disclosure for use in the treatment of a disease, particularly a human disease, more particularly a human disease selected from cancer, an inflammatory and an autoimmune disease, wherein at least one of said cognate pairs of VL and VH domains, or, when present, of said first, second, third, or fourth scFv fragment is able to specifically interact with a target of therapeutic relevance in the corresponding disease.

In one example the present disclosure describes a method for treating a patient suffering from a disease, particularly a human disease, more particularly a human disease selected from cancer, an inflammatory and an autoimmune disease, comprising administering to a subject an effective amount of the hetero-dimeric protein of the present disclosure, wherein at least one of said cognate pairs of VL and VH domains, or of said third, fourth, fifth, or sixth functional domain is able to specifically interact with a target of therapeutic relevance in the corresponding disease.

[0017] Particular embodiments of the present invention are set forth in the appended dependent claims.

FIGURES

Figure 1 shows a schematic representation of Assembly 1 (see Example 1).

Figure 2 shows a schematic representation of Assembly 3 (see Example 1).

CLAIMS

1. A hetero-dimeric protein comprising a first and a second single-chain protein,
wherein said first single-chain protein comprises a first amino acid sequence
consisting of (from the N- to the C-terminus):

- 5 (ia) a first VL domain,
(iia) a first polypeptide linker,
(iiia) a second VL domain,
(iva) optionally, a first scFv fragment that is fused via a third polypeptide linker
to said first VL domain, and
10 (va) optionally, a second scFv fragment that is fused via a fourth polypeptide
linker to said second VL domain, and

wherein said second single-chain protein comprises a second amino acid
sequence consisting of (from the N- to the C-terminus):

- (ib) a first VH domain,
15 (iib) a second polypeptide linker,
(iiib) a second VH domain,
(ivb) optionally, a third scFv fragment that is fused via a fifth polypeptide linker
to said first VH domain, and
(vb) optionally, a fourth scFv fragment that is fused via a sixth polypeptide linker
20 to said second VH domain, and

provided that at least one of said first, second, third and fourth scFv fragments is
present,

wherein at least one of said VL domains comprises (i) human V κ framework
regions I to III; (ii) CDR domains CDR1, CDR2 and CDR3; and (iii) a framework
25 region IV, which is a human V λ -based sequence for framework region IV.

2. The hetero-dimeric protein of claim 1, wherein

- (a) a second of said first, second, third and fourth scFv fragments is present;

(b) a second and a third of said first, second, third and fourth scFv fragments is present; or

(c) said first, second, third and fourth scFv fragments are all present.

3. The hetero-dimeric protein of claim 1 or 2, wherein said first polypeptide linker consists of from 5 to 20 amino acid residues, particularly from 6 to 15 amino acid residues.

4. The hetero-dimeric protein of any one of claims 1 to 3, wherein (a) said first VL domain (ia) and said first VH domain (ib) form a first cognate pair of variable domains with specificity to a first target antigen, and said second VL domain (iia) and said second VH domain (iib) form a second cognate pair of variable domains with specificity to a second target antigen; or (b) said first VL domain (ia) and said second VH domain (iib) form a first cognate pair of variable domains with specificity to a first target antigen, and said second VL domain (iia) and said first VH domain (ib) form a second cognate pair of variable domains with specificity to a second target antigen.

5. The hetero-dimeric protein of any one of claims 1 to 4, wherein at least one of said VL, VH or scFv domains comprises CDR regions derived from a parental rabbit antibody.

6. The hetero-dimeric protein of any one of claims 1 to 5, wherein the cognate pair of one of said first and said second VL and VH domains is specific for an antigen selected from the list of: a cancer target; and a target present on immune effector cells, in particular CD3.

7. A nucleic acid sequence or two nucleic acid sequences encoding the first and the second single-chain proteins of the hetero-dimeric protein of any one of claims 1 to 6.

8. A vector or two vectors comprising the nucleic acid sequence or the two nucleic acid sequences of claim 7.

9. A host cell or host cells comprising the vector or the two vectors of claim 8.

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10. A method for producing the hetero-dimeric protein of any one of claims 1 to 6, or the first and the second single-chain proteins of said hetero-dimeric protein, comprising (i) providing the nucleic acid sequence or the two nucleic acid sequences of claim 7, or the vector or the two vectors of claim 8, expressing said nucleic acid sequence or nucleic acid sequences, or said vector or vectors, and collecting said hetero-dimeric protein from the expression system, or (ii) providing a host cell or host cells according to claim 9, culturing said host cell or said host cells; and collecting said first and second single-chain proteins, or said hetero-dimeric protein, from the cell culture.

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11. A pharmaceutical composition comprising the hetero-dimeric protein of any one of claims 1 to 6 and a pharmaceutically acceptable carrier.

12. The hetero-dimeric protein of any one of claims 1 to 6 for use in the treatment of a disease, particularly a human disease, more particularly a human disease selected from cancer, an inflammatory and an autoimmune disease, wherein at least one of said cognate pairs of VL and VH domains, or, when present, of said first, second, third, or fourth scFv fragment, is able to specifically interact with a target of therapeutic relevance in the corresponding disease.

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