

not appropriate as a basis for easily developing recombinant, tri-or tetraspecific antibodies against three or four antigens starting from two antibodies against the first and the second antigen, as either the heavy chains of these antibodies and/or the identical light chains have to be optimized first and then further antigen binding peptides against the third and fourth antigen have to be added.

WO 2006/093794 relates to heterodimeric protein binding compositions. WO 99/37791 describes multipurpose antibody derivatives. Morrison, S.L., et al., J. Immunol. 160 (1998) 2802-2808 refers to the influence of variable region domain exchange on the functional properties of IgG.

WO 2013/02362 relates to heterodimerized polypeptides. WO 2013/12733 relates to polypeptides comprising heterodimeric Fc regions. WO 2012/131555 relates to engineered hetero-dimeric immunoglobulins. EP 2647707 relates to engineered hetero-dimeric immunoglobulins.

WO 2013/026835 relates to bispecific, Fc free antibodies with a domain crossover. WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254 and Schaefer, W. et al, PNAS, 108 (2011) 11187-1191 relate to bivalent, bispecific IgG antibodies with a domain crossover.

The multispecific antibodies with VH/VL replacement/exchange in one binding to prevent light chain mispairing (CrossMab^{VH-VL}) which are described in WO2009/080252, (see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191) clearly reduce the byproducts caused by the mismatch of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange). However their preparation is not completely free of side products. The main side product is based on a Bence-Jones -type interaction -see also Schaefer, W. et al, PNAS, 108 (2011) 11187-1191; in Fig. S1I of the Supplement).

Therefore there is still a need for further reduction of such side products improve e.g. the yield of such bispecific antibodies.

Summary

The disclosure, in a first aspect, relates to a multispecific antibody, comprising:

a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and

5 b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

10 i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or

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20 ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

The disclosure, in another aspect, relates to a multispecific antibody, comprising:

25 a) a first light chain and a first heavy chain of a first antibody which specifically binds to a first antigen; and

30 b) a second light chain and a second heavy chain of a second antibody which specifically binds to a second antigen, and wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

wherein

5 in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

10 The disclosure, in another aspect, relates to a multispecific antibody according to any one of the preceding claims that specifically binds to human TWEAK and that specifically binds to human IL17, wherein

A) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:24, and a variable light chain domain (VL) of SEQ ID NO:25; and

B) the multispecific antibody comprises a variable heavy chain domain (VH) of SEQ ID NO:26, and a variable light chain domain (VL) of SEQ ID NO:27.

15 A further aspect of the disclosure is a method for the preparation of a multispecific antibody according to the disclosure

comprising the steps of

A) transforming a host cell with vectors comprising nucleic acid molecules encoding

20 a) the first light chain and the first heavy chain of a first antibody which specifically binds to a first antigen; and

b) the second light chain and the second heavy chain of a second antibody which specifically binds to a second antigen, wherein the variable domains VL and VH in the second light chain and second heavy chain of the second antibody are replaced by each other; and

5 wherein

i) in the constant domain CL of the first light chain under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein
10 in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or

ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and wherein
15 in the constant domain CH1 of the second heavy chain under b) the amino acid at position 147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D)
20 (numbering according to Kabat EU index).

B) culturing the host cell under conditions that allow synthesis of said antibody molecule; and

C) recovering said antibody molecule from said culture.

25 A further aspect of the disclosure is a nucleic acid encoding the amino acid sequences of a multispecific antibody according to the disclosure.

A further aspect of the disclosure are expression vectors containing the nucleic acid according to the disclosure capable of expressing said nucleic acid in a host cell.

A further embodiment of the disclosure is a host cell comprising a vector according to the disclosure.

5 A further aspect of the disclosure is a composition comprising the antibody according to the disclosure. The composition may be, e.g. a pharmaceutical or a diagnostic composition.

A further aspect of the disclosure is a pharmaceutical composition comprising an antibody according to the disclosure and at least one pharmaceutically acceptable excipient.

10 A further embodiment of the disclosure is a method for the treatment of a patient in need of therapy, characterized by administering to the patient a therapeutically effective amount of an antibody according to the disclosure.

15 According to the disclosure, the ratio of a desired multispecific antibody compared to undesired main side Bence Jones-type products can be improved by the introduction of substitutions of charged amino acids with the opposite charges at specific amino acid positions in the CH1 and CL domains.

Description of the Figures

Figure 1 Some examples of multispecific antibodies according to the invention with VH/VL domain replacement in one antibody binding arm and specific mutations in one CH1/CL domain interface:

20 at least the amino acid at position 124 of the CL domain is substituted independently by lysine (K) , arginine (R) or Histidine (H) (numbering according to Kabat), and

25 at least the amino acid at position 147 of the CH1 domain or the amino acid at position 213 of the CH1 domain is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

Figure 1A: VH/VL domain replacement in one antibody binding arm and specific mutations in the CH1/CL domain interface of

30 the other antibody binding arm.