domain antibodies (Wu et al., Nat Biotechnol 25, 1290-1297 (2007)), or bivalent rat/mouse hybrid bispecific IgGs (see e.g. Lindhofer et al., J Immunol 155, 219-225 (1995)).

Also several bispecific formats wherein the antibody core structure (IgA, IgD, IgE, IgG or IgM) is no longer retained have been made. Examples include diabodies (see e.g. Holliger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1995)), tandem scFv molecules (see e.g. Bargou et al., Science 321, 974-977 (2008)), and various derivatives thereof.

The multitude of formats that are being developed shows the great potential attributed to bispecific antibodies. The task of generating bispecific antibodies suitable for a particular purpose is, however, by no means trivial and subject to a number of considerations. For example, the valency and geometry of the antibody needs to be appropriately chosen, depending on the characteristics of the target antigens and the intended effect. As for all therapeutic antibodies, efficacy and toxicity have to be balanced, which requires i.a. minimization of immunogenicity and optimization of pharmacokinetic properties of the antibody. Also, the desirablility of Fc-mediated effects has to be considered. Furthermore, the production of bispecific antibody constructs at a clinically sufficient quantity and purity poses a major challenge, as the homodimerization of antibody heavy chains and/or the mispairing of antibody heavy and light chains of different specificities upon co-expression decreases the yield of the correctly assembled construct and results in a number of non-functional side products from which the desired bispecific antibody may be difficult to separate.

Given the increasing number of possible applications of bispecific antibodies, and the difficulties and disadvantages associated with currently available bispecific antibodies, there remains a need for novel, improved formats of such molecules.

Summary ~~of the Invention~~

~~In a first aspect, the invention~~The present disclosure provides a bispecific antigen binding molecule, comprising a first Fab fragment which specifically binds to a first antigen, a second Fab fragment which specifically binds to a second antigen, and an Fc domain composed of a first and a second subunit capable of stable association; wherein

a) the bispecific antigen binding molecule provides monovalent binding to the first and/or the second antigen,

b) the first Fab fragment, the second Fab fragment and the first Fc domain subunit are fused to each other, and

c) in the first and/or the second Fab fragment one of the following replacements is made: (i) the variable domains VL and VH are replaced by each other, (ii) the constant domains CL and CH1 are replaced by each other, or (iii) both the variable and constant domains VL-CL and VH-CH1 are replaced by each other,

provided that not the same replacement is made in the first and the second Fab fragment.

In one aspect, there is provided a bispecific antigen binding molecule, comprising a first Fab fragment which specifically binds to a first antigen, a second Fab fragment which specifically binds to a second antigen, and an Fc domain composed of a first and a second subunit capable of stable association; wherein

a) the bispecific antigen binding molecule provides monovalent binding to the first and/or the second antigen,

b) the first Fab fragment, the second Fab fragment and the first Fc domain subunit are fused to each other,

c) in the first and/or the second Fab fragment one of the following replacements is made: (i) the variable domains VL and VH are replaced by each other, or (ii) the constant domains CL and CH1 are replaced by each other,

provided that not the same replacement is made in the first and the second Fab fragment, and

d) the bispecific antigen binding molecule does not comprise a single chain Fab fragment.

In particular embodiments, the first Fab fragment is fused at its C-terminus to the N-terminus of the second Fab fragment, which is in turn fused at its C-terminus to the N-terminus of the first Fc domain subunit. In a more specific embodiment, the first Fab fragment is fused at the C-terminus of its heavy chain to the N-terminus of the heavy chain of the second Fab fragment, which is in turn fused at the C-terminus of its heavy chain to the N-terminus of the first Fc domain subunit. In other embodiments, the second Fab fragment is fused at its C-terminus to the N-terminus of the first Fab fragment, which is in turn fused at its C-terminus to the N-terminus of the first Fc domain subunit. In a more specific embodiment, the second Fab fragment is fused at the C-terminus of its heavy chain to the N-terminus of the heavy chain of the first Fab fragment, which is in turn fused at the C-terminus of its heavy chain to the N-terminus of the first Fc domain subunit. In still other embodiments, the second Fab fragment is fused at its C-terminus to the N-terminus of the first Fc domain subunit, which is in turn fused at its C-terminus to the N-

terminus of the first Fab fragment. In a more specific embodiment, the second Fab fragment is fused at the C-terminus of its heavy chain to the N-terminus of the first Fc domain subunit, which is in turn fused at its C-terminus to the N-terminus of the heavy chain of the first Fab fragment.

In embodiments wherein either the first Fab fragment is fused at the C-terminus of its heavy chain to the N-terminus of the heavy chain of the second Fab fragment which is in turn fused at the C-terminus of its heavy chain to the N-terminus of the first Fc domain subunit, or the second Fab fragment is fused at the C-terminus of its heavy chain to the N-terminus of the heavy chain of the first Fab fragment which is in turn fused at the C-terminus of its heavy chain to the N-terminus of the first Fc domain subunit, additionally the Fab light chain of the first Fab fragment and the Fab light chain of the second Fab fragment may be fused to each other, optionally via a peptide linker.

In one embodiment, the replacement is made in the first Fab fragment. In some embodiments, the replacement is a replacement of the variable domains VL and VH by each other. In other embodiments the replacement is a replacement of the constant domains CL and CH1 by each other.

In one embodiment the bispecific antigen binding molecule essentially consists of the first Fab fragment, the second Fab fragment, the Fc domain, and optionally one or more peptide linkers.

In particular embodiments, the bispecific antigen binding molecule comprises a third Fab fragment which specifically binds to the first or the second antigen. In one embodiment, the third Fab fragment is fused to the second Fc domain subunit. In a more specific embodiment, the third Fab fragment is fused at its C-terminus to the N-terminus of the second Fc domain subunit. In ~~en~~ an even more specific embodiment, the third Fab fragment is fused at the C-terminus of its heavy chain to the N-terminus of the second Fc domain subunit. In one embodiment, the third Fab fragment specifically binds to the second antigen. In some embodiments the ~~the~~ second Fab fragment, the third Fab fragment and the Fc domain are part of an immunoglobulin molecule. In a specific such embodiment, the immunoglobulin molecule is an IgG class immunoglobulin molecule, more specifically an IgG1 or IgG4 subclass immunoglobulin molecule. In one embodiment, the immunoglobulin molecule is a human immunoglobulin molecule. In one embodiment, the bispecific antigen binding molecule essentially consists of a first Fab fragment which specifically binds to the first antigen, an immunoglobulin molecule which specifically binds to the second antigen, and optionally one or more peptide linkers.

In one embodiment, the same replacement is made in Fab fragments that specifically bind to the same antigen. In a further embodiment, a replacement is made only in the first Fab fragment. In one embodiment, the bispecific antigen binding molecule provides monovalent binding to the first antigen. In one embodiment, the bispecific antigen binding molecule does not comprise a single-chain Fab fragment.

In certain embodiments, the Fc domain comprises a modification promoting the association of the first and second Fc domain subunit. In a specific such embodiment, an amino acid residue in the CH3 domain of the first subunit of the Fc domain is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and an amino acid residue in the CH3 domain of the second subunit of the Fc domain is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. In one embodiment, the Fc domain is an IgG Fc domain, specifically an IgG1 or IgG4 Fc domain. In one embodiment the Fc domain is human. In certain embodiments, the Fc domain is engineered to have altered binding affinity to an Fc receptor and/or altered effector function, as compared to a non-engineered Fc domain.

According to another aspect, ~~of the invention~~ there is provided an isolated polynucleotide encoding a bispecific antigen binding molecule ~~of the invention or a fragment thereof~~as defined herein. The ~~invention~~disclosure also encompasses polypeptides encoded by the polynucleotides ~~of the invention~~as defined herein. ~~The invention further~~In another aspect, there is provides~~d~~ an expression vector comprising the isolated polynucleotide ~~of the invention, and~~as defined herein. In another aspect, there is provided a host cell comprising the isolated polynucleotide or the expression vector ~~of the invention~~as defined herein. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell. ~~In another aspect~~The disclosure ~~is~~ provide~~d~~s a method of producing the bispecific antigen binding molecule ~~of the invention~~, comprising the steps of a) culturing the host cell ~~of the invention~~as defined herein under conditions suitable for the expression of the bispecific antigen binding molecule and b) recovering the bispecific antigen binding molecule. In another aspect, there is provided a method for producing a bispecific antigen binding molecule, comprising the steps of a) culturing the host cell as defined above under conditions suitable for the expression of the bispecific antigen binding molecule and b) recovering the bispecific antigen binding molecule. The ~~invention~~disclosure also encompasses a bispecific antigen binding molecule produced by the method ~~of the invention~~as described herein. ~~The invention further~~In another aspect, there is provides~~d~~ a pharmaceutical composition comprising the bispecific antigen binding molecule ~~of the invention~~as defined herein and a pharmaceutically acceptable carrier.

Also encompassed by the ~~invention~~disclosure are methods of using the bispecific antigen binding molecule and pharmaceutical composition ~~of the invention~~as defined herein. In ~~one~~another aspect, ~~the invention~~there is provides~~d~~ a bispecific antigen binding molecule or a pharmaceutical composition ~~of the invention~~as defined herein for use as a medicament. In ~~one~~another aspect, there is provided a bispecific antigen binding molecule or a pharmaceutical composition ~~according to the invention~~as defined herein for use in the treatment of a disease in an individual in need thereof. In a specific embodiment the disease is cancer.

~~Also~~In another aspect, there is provided ~~is~~ the use of a bispecific antigen binding molecule ~~of the invention~~as defined herein for the manufacture of a medicament for the treatment of a disease in an individual in need thereof~~; as well as~~. The disclosure also provides a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the bispecific antigen binding molecule ~~according to the~~

~~invention~~as defined herein in a pharmaceutically acceptable form. In a specific embodiment the disease is cancer. In any of the above embodiments the individual preferably is a mammal, particularly a human.

Brief Description of the Drawings

FIGURE 1. Illustration of exemplary formats of the bispecific antigen binding molecules of the invention. (A) "2+1" format, with Crossfab fragment of different specificity fused to N-terminus of a Fab fragment comprised in an antibody ("2+1 IgG Crossfab (N-terminal)"). (B) "1+1" format, with Crossfab fragment of different specificity fused to N-terminus of a Fab fragment