cell activation and re-direction that combine good efficacy and produceability with low toxicity and favorable pharmacokinetic properties.

Summary ~~of the Invention~~

The present disclosure~~In a first aspect the present invention~~ provides a T cell activating bispecific antigen binding molecule comprising a first and a second antigen binding moiety, one of which is a Fab molecule capable of specific binding to an activating T cell antigen and the other one of which is a Fab molecule capable of specific binding to a target cell antigen, and an Fc domain composed of a first and a second subunit capable of stable association; wherein the first antigen binding moiety is (a) a single chain Fab molecule wherein the Fab light chain and the Fab heavy chain are connected by a peptide linker, or (b) a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged.

In one aspect, there is provided a T cell activating bispecific antigen binding molecule comprising a first and a second antigen binding moiety, one of which is a Fab molecule capable of specific binding to an activating T cell antigen and the other one of which is a Fab molecule capable of specific binding to a target cell antigen, and an Fc domain composed of a first and a second subunit capable of stable association;
wherein the first antigen binding moiety is

(a) a single chain Fab molecule wherein the Fab light chain and the Fab heavy chain are connected by a peptide linker, or

(b) a crossover Fab molecule wherein either the variable or the constant regions of the Fab light chain and the Fab heavy chain are exchanged, and

wherein the first and the second antigen binding moiety are fused to each other, optionally via a peptide linker.

In a particular embodiment, not more than one antigen binding moiety capable of specific binding to an activating T cell antigen is present in the T cell activating bispecific antigen binding molecule (i.e. the T cell activating bispecific antigen binding molecule provides monovalent binding to the activating T cell antigen). In particular embodiments, the first antigen binding moiety is a crossover Fab molecule. In even more particular embodiments, the first antigen binding moiety is a crossover Fab molecule wherein the constant regions of the Fab light chain and the Fab heavy chain are exchanged.

In some embodiments, the first and the second antigen binding moiety of the T cell activating bispecific antigen binding molecule are fused to each other, optionally via a peptide linker. In one such embodiment, the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety. In another such embodiment, the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety. In yet another such embodiment, the second antigen binding moiety is fused at the C-terminus of the Fab light chain to the N-terminus of the Fab light chain of the first antigen binding moiety. In embodiments wherein the first antigen binding moiety is a crossover Fab molecule and wherein either (i) the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety or (ii) the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety, additionally the Fab light chain of the first

antigen binding moiety and the Fab light chain of the second antigen binding moiety may be fused to each other, optionally via a peptide linker.

In one embodiment, the second antigen binding moiety of the T cell activating bispecific antigen binding molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the second subunit of the Fc domain. In another embodiment, the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain.

In one embodiment, the first and the second antigen binding moiety of the T cell activating bispecific antigen binding molecule are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain.

In certain embodiments, the T cell activating bispecific antigen binding molecule comprises a third antigen binding moiety which is a Fab molecule capable of specific binding to a target cell antigen. In one such embodiment, the third antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or second subunit of the Fc domain. In a particular embodiment, the second and the third antigen binding moiety of the T cell activating antigen binding molecule are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety. In another particular embodiment, the first and the third antigen binding moiety of the T cell activating antigen binding molecule are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain, and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety. The components of the T cell activating bispecific antigen binding molecule may be fused directly or through suitable peptide linkers. In one embodiment the second and the third antigen binding moiety and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG_1 subclass immunoglobulin. In another embodiment, the immunoglobulin is an IgG_4 subclass immunoglobulin.

In another aspect, there is provided a T cell activating bispecific antigen binding molecule comprising a first antigen binding moiety, a second antigen binding moiety, a third antigen binding moiety, and an Fc domain composed of a first and a second subunit capable of stable association, wherein

(i) the first antigen binding moiety is a Fab molecule capable of specific binding to an activating T cell antigen, and the second and the third antigen binding moiety are each a Fab molecule capable of specific binding to a target cell antigen,

(ii) the first antigen binding moiety is a crossover Fab molecule wherein either the variable or the constant regions, particularly the constant regions, of the Fab light chain and the Fab heavy chain are exchanged; and

(iii) the second and the third antigen binding moiety are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain, and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second antigen binding moiety, or

the first and the third antigen binding moiety are each fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain, and the second antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first antigen binding moiety.

In a particular embodiment, the Fc domain is an IgG Fc domain. In a specific embodiment, the Fc domain is an IgG_1 Fc domain. In another specific embodiment, the Fc domain is an IgG_4 Fc domain. In an even more specific embodiment, the Fc domain is an IgG_4 Fc domain comprising

embodiment, the target cell antigen is selected from the group consisting of: Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Carcinoembryonic Antigen (CEA), Fibroblast Activation Protein (FAP), CD19, CD20 and CD33.

According to another aspect, ~~of the invention~~ there is provided an isolated polynucleotide encoding a T cell activating bispecific antigen binding molecule ~~of the invention~~as defined herein or a fragment thereof. The ~~invention~~disclosure also encompasses polypeptides encoded by the polynucleotides ~~of the invention~~as defined herein. The ~~invention~~disclosure further provides an expression vector comprising the isolated polynucleotide as defined herein. In another aspect, there is provided a vector, particularly an expression vector, comprising the isolated polynucleotide as defined herein. In another aspect, there is provided~~of the invention, and~~ 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, there is provided a method of producing the T cell activating bispecific antigen binding molecule ~~of the invention~~as defined herein, comprising the steps of a) culturing the host cell ~~of the invention~~as defined herein under conditions suitable for the expression of the T cell activating bispecific antigen binding molecule and b) recovering the T cell activating bispecific antigen binding molecule. The ~~invention~~disclosure also encompasses a T cell activating bispecific antigen binding molecule produced by the method ~~of the invention~~as defined herein.

In another aspect, there is provided~~The invention further provides~~ a pharmaceutical composition comprising the T cell activating 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 T cell activating bispecific antigen binding molecule and pharmaceutical composition ~~of the invention~~as defined herein. In another~~one~~ aspect, there is provided~~the invention provides~~ a T cell activating bispecific antigen binding molecule or a pharmaceutical composition ~~of the invention~~as defined herein for use as a medicament. In another~~one~~ aspect, there is provided a T cell activating bispecific antigen binding molecule or a pharmaceutical composition as defined herein~~according to the invention~~ for use in the treatment of a disease in an individual in need thereof. In a specific embodiment the disease is cancer.

Also provided is the use of a T cell activating 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 a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the T cell activating bispecific antigen binding molecule as defined herein~~according to the invention~~ 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.

In another aspect, there is provided the use of the T cell activating bispecific antigen binding molecule as defined herein for the manufacture of a medicament for the treatment of cancer.

The ~~invention~~disclosure also provides a method for inducing lysis of a target cell, particularly a tumor cell, comprising contacting a target cell with a T cell activating bispecific antigen binding molecule ~~of the invention~~as defined herein in the presence of a T cell, particularly a cytotoxic T cell.

In another aspect, there is provided a T cell activating bispecific antigen binding molecule as defined herein for use in inducing lysis of a target cell, wherein said target cell is contacted with said T cell activating bispecific antigen binding molecule in the presence of a T cell.

Brief Description of the Drawings

FIGURE 1. Exemplary configurations of the T cell activating bispecific antigen binding molecules of the invention. Illustration of (A) the "1+1 IgG scFab, one armed", and (B) the "1+1 IgG scFab, one armed inverted" molecule. In the "1+1 IgG scFab, one armed" molecule the light chain of the T cell targeting Fab is fused to the heavy chain by a linker, while the "1+1 IgG scFab, one armed inverted" molecule has the linker in the tumor targeting Fab. (C) Illustration of the "2+1 IgG scFab" molecule. (D) Illustration of the "1+1 IgG scFab" molecule. (E) Illustration of the "1+1 IgG Crossfab" molecule. (F) Illustration of the "2+1 IgG Crossfab" molecule. (G) Illustration of the "2+1 IgG Crossfab" molecule with alternative order of Crossfab and Fab components ("inverted"). (H) Illustration of the "1+1 IgG Crossfab light chain (LC) fusion" molecule. (I) Illustration of the "1+1 CrossMab" molecule. (J) Illustration of the "2+1 IgG Crossfab, linked light chain" molecule. (K) Illustration of the "1+1 IgG Crossfab, linked light chain" molecule. (L) Illustration of the "2+1 IgG Crossfab, inverted, linked light chain" molecule. (M) Illustration of the "1+1 IgG Crossfab, inverted, linked light chain" molecule. Black dot: optional modification in the Fc domain promoting heterodimerization.

FIGURE 2. SDS PAGE (4-12% Bis/Tris, NuPage Invitrogen, Coomassie-stained) of "1+1 IgG scFab, one armed" (anti-MCSP/anti-huCD3) (see SEQ ID NOs 1, 3, 5), non reduced (A) and reduced (B), and of "1+1 IgG scFab, one armed inverted" (anti-MCSP/anti-huCD3) (see SEQ ID NOs 7, 9, 11), non reduced (C) and reduced (D).

FIGURE 3. Analytical size exclusion chromatography (Superdex 200 10/300 GL GE Healthcare; 2 mM MOPS pH 7.3, 150 mM NaCl, 0.02% (w/v) NaCl; 50 µg sample injected) of "1+1 IgG scFab, one armed" (anti-MCSP/anti-huCD3) (see SEQ ID NOs 1, 3, 5) (A) and "1+1 IgG scFab, one armed inverted" (anti-MCSP/anti-huCD3) (see SEQ ID NOs 7, 9, 11) (B).

FIGURE 4. SDS PAGE (4-12% Bis/Tris, NuPage Invitrogen, Coomassie-stained) of "1+1 IgG

scFab, one armed" (anti-EGFR/anti-huCD3) (see SEQ ID NOs 43, 45, 57), non reduced (A) and reduced (B), and of "1+1 IgG scFab, one armed inverted" (anti-EGFR/anti-huCD3) (see SEQ ID NOs 11, 49, 51), non reduced (C) and reduced (D).