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- (71) **Applicant (for all designated States except US):** **F. HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, 4070 Basel (CH).
- (71) **Applicant (for US only):** **HOFFMANN-LA ROCHE INC.** [US/US]; Overlook at Great Notch, 150 Clove Road, 8th Floor, Suite 8 - Legal Department, Little Falls, New Jersey 07424 (US).
- (72) **Inventors:** **FREIMOSER-GRUNDSCHÖBER, Anne;**
c/o Roche Glycart AG, Wagistrasse 18, 8952 Schlieren

(CH). **IMHOF-JUNG, Sabine;** c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **KLEIN, Christian;** c/o Roche Glycart AG, Wagistrasse 18, 8952 Schlieren (CH). **KLOSTERMANN, Stefan;** c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **LIFKE, Valeria;** c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **MOLHOJ, Michael;** c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **REGULA, Joerg Thomas;** c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **SCHAEFER, Wolfgang;** c/o Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg (DE). **UMAÑA, Pablo;** c/o Roche Glycart AG, Wagistrasse 18, 8952 Schlieren (CH).

(74) **Agent:** **CUENI, Leah;** Grenzacherstrasse 124, 4070 Basel (CH).

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(54) **Title:** ANTI-CD3XTIM-3 BISPECIFIC T CELL ACTIVATING ANTIGEN BINDING MOLECULES

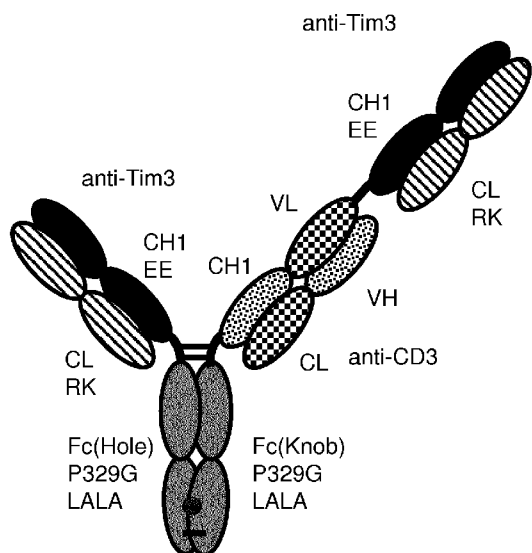


Figure 2

(57) **Abstract:** The present invention generally relates to novel bispecific antigen binding molecules for T cell activation and re-direction to specific target cells. In addition, the present invention relates to polynucleotides encoding such bispecific antigen binding molecules, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the bispecific antigen binding molecules of the invention, and to methods of using these bispecific antigen binding molecules in the treatment of disease.



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ANTI-CD3XTIM-3 BISPECIFIC T CELL ACTIVATING ANTIGEN BINDING MOLECULES

Field of the Invention

The present invention generally relates to bispecific antigen binding molecules for activating T cells. In addition, the present invention relates to polynucleotides encoding such bispecific antigen binding molecules, and vectors and host cells comprising such polynucleotides. The invention further relates to methods for producing the bispecific antigen binding molecules of the invention, and to methods of using these bispecific antigen binding molecules in the treatment of disease.

Background

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged.

An attractive way of achieving this is by inducing an immune response against the tumor, to make immune effector cells such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs) attack and destroy tumor cells. CTLs constitute the most potent effector cells of the immune system, however they cannot be activated by the effector mechanism mediated by the Fc domain of conventional therapeutic antibodies.

In this regard, bispecific antibodies designed to bind with one “arm” to a surface antigen on target cells, and with the second “arm” to an activating, invariant component of the T cell receptor (TCR) complex, have become of interest in recent years. The simultaneous binding of such an antibody to both of its targets will force a temporary interaction between target cell and T cell, causing activation of any cytotoxic T cell and subsequent lysis of the target cell. Hence, the immune response is re-directed to the target cells and is independent of peptide antigen presentation by the target cell or the specificity of the T cell as would be relevant for normal MHC-restricted activation of CTLs. In this context it is crucial that CTLs are only activated when a target cell is presenting the bispecific antibody to them, i.e. the immunological synapse is mimicked. Particularly desirable are bispecific antibodies that do not require lymphocyte preconditioning or co-stimulation in order to elicit efficient lysis of target cells.

Several bispecific antibody formats have been developed and their suitability for T cell mediated immunotherapy investigated. Out of these, the so-called BiTE (bispecific T cell engager) molecules have been very well characterized and already shown some promise in the clinic (reviewed in Nagorsen and B  uerle, *Exp Cell Res* 317, 1255-1260 (2011)). BiTEs are tandem
5 scFv molecules wherein two scFv molecules are fused by a flexible linker. Further bispecific formats being evaluated for T cell engagement include diabodies (Holliger et al., *Prot Eng* 9, 299-305 (1996)) and derivatives thereof, such as tandem diabodies (Kipriyanov et al., *J Mol Biol* 293, 41-66 (1999)). A more recent development are the so-called DART (dual affinity retargeting) molecules, which are based on the diabody format but feature a C-terminal disulfide
10 bridge for additional stabilization (Moore et al., *Blood* 117, 4542-51 (2011)). The so-called triomabs, which are whole hybrid mouse/rat IgG molecules and also currently being evaluated in clinical trials, represent a larger sized format (reviewed in Seimetz et al., *Cancer Treat Rev* 36, 458-467 (2010)).

The variety of formats that are being developed shows the great potential attributed to T cell re-
15 direction and activation in immunotherapy. The task of generating bispecific antibodies suitable therefor is, however, by no means trivial, but involves a number of challenges that have to be met related to efficacy, toxicity, applicability and produceability of the antibodies.

Small constructs such as, for example, BiTE molecules – while being able to efficiently crosslink effector and target cells – have a very short serum half life requiring them to be administered to
20 patients by continuous infusion. IgG-like formats on the other hand – while having the great benefit of a long half life – suffer from toxicity associated with the native effector functions inherent to IgG molecules. Their immunogenic potential constitutes another unfavorable feature of IgG-like bispecific antibodies, especially non-human formats, for successful therapeutic development. Finally, a major challenge in the general development of bispecific antibodies has
25 been the production of bispecific antibody constructs at a clinically sufficient quantity and purity, due to the mispairing of antibody heavy and light chains of different specificities upon co-expression, which 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.

30 Different approaches have been taken to overcome the chain association issue in bispecific antibodies (see e.g. Klein et al., *mAbs* 6, 653-663 (2012)). For example, the ‘knobs-into-holes’ strategy aims at forcing the pairing of two different antibody heavy chains by introducing mutations into the CH3 domains to modify the contact interface. On one chain bulky amino acids

are replaced by amino acids with short side chains to create a 'hole'. Conversely, amino acids with large side chains are introduced into the other CH3 domain, to create a 'knob'. By coexpressing these two heavy chains (and two identical light chains, which have to be appropriate for both heavy chains), high yields of heterodimer ('knob-hole') versus homodimer ('hole-hole' or 'knob-knob') are observed (Ridgway, J.B., et al., Protein Eng. 9 (1996) 617-621; and WO 96/027011). The percentage of heterodimer could be further increased by remodeling the interaction surfaces of the two CH3 domains using a phage display approach and the introduction of a disulfide bridge to stabilize the heterodimers (Merchant, A.M., et al., Nature Biotech. 16 (1998) 677-681; Atwell, S., et al., J. Mol. Biol. 270 (1997) 26-35). New approaches for the knobs-into-holes technology are described in e.g. in EP 1870459 A1.

The 'knobs-into-holes' strategy does, however, not solve the problem of heavy chain-light chain mispairing, which occurs in bispecific antibodies comprising different light chains for binding to the different target antigens.

A strategy to prevent heavy chain-light chain mispairing is to exchange domains between the heavy and light chains of one of the binding arms of a bispecific antibody (see WO 2009/080251, WO 2009/080252, WO 2009/080253, WO 2009/080254 and Schaefer, W. et al, PNAS, 108 (2011) 11187-11191, which relate to bispecific IgG antibodies with a domain crossover).

Exchanging the heavy and light chain variable domains VH and VL in one of the binding arms of the bispecific antibody (WO2009/080252, see also Schaefer, W. et al, PNAS, 108 (2011) 11187-11191) clearly reduces the side products caused by the mispairing of a light chain against a first antigen with the wrong heavy chain against the second antigen (compared to approaches without such domain exchange). Nevertheless, these antibody preparations are not completely free of side products. The main side product is based on a Bence Jones-type interaction (Schaefer, W. et al, PNAS, 108 (2011) 11187-11191; in Fig. S1I of the Supplement). A further reduction of such side products is thus desirable to improve e.g. the yield of such bispecific antibodies.

The choice of target antigens and appropriate binders for both the T cell antigen and the target cell antigen is a further crucial aspect in the generation of T cell bispecific (TCB) antibodies for therapeutic application.

Tim-3 is a human protein which belongs to the immunoglobulin superfamily, and Tim family of proteins. In humans, as similar to mice, Tim-3 is expressed on T-cells as well as phagocytic cells such as macrophages and dendritic cells. Tim-3 is also associated with cancer, in particular, with cancer stem cells.

The present invention provides novel, improved bispecific antigen binding molecules designed for T cell activation and re-direction, targeting Tim-3 and an activating T cell antigen such as CD3, that combine good efficacy and produceability with low toxicity and favorable pharmacokinetic properties.

5

Summary of the Invention

The present inventors have developed a novel T cell activating bispecific antigen binding molecule with unexpected, improved properties targeting Tim-3.

Thus, the present invention provides a T cell activating bispecific antigen binding molecule comprising

- 10 (a) a first antigen binding moiety which specifically binds to a first antigen;
(b) a second antigen binding moiety which specifically binds to a second antigen;
wherein the first antigen is an activating T cell antigen and the second antigen is Tim-3, or the first antigen is Tim-3 and the second antigen is an activating T cell antigen.

In one aspect the present invention provides a T cell activating bispecific antigen binding
15 molecule comprising

- (a) a first antigen binding moiety which specifically binds to a first antigen;
(b) a second antigen binding moiety which specifically binds to a second antigen;
wherein the first antigen is an activating T cell antigen and the second antigen is Tim-3, or the first antigen is Tim-3 and the second antigen is an activating T cell antigen; and
20 wherein the antigen binding moiety which specifically binds to Tim-3 comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3
25 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region,
30 particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain

variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

In one embodiment, the antigen binding moiety which specifically binds to Tim-3 comprises (i) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 20 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 21, (ii) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 34, (iii) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36, (iv) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 43 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 44, or (v) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46.

In particular embodiments, the first and/or the second antigen binding moiety is a Fab molecule. In a particular embodiment, the second antigen binding moiety is a Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other (i.e. according to such embodiment, the second Fab molecule is a crossover Fab molecule wherein the variable or constant domains of the Fab light chain and the Fab heavy chain are exchanged).

In particular embodiments, the first (and the third, if any) Fab molecule is a conventional Fab molecule. In a further particular embodiment, not more than one Fab molecule 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 one embodiment, the first antigen is Tim-3 and the second antigen is an activating T cell antigen. In a more specific embodiment, the activating T cell antigen is CD3, particularly CD3
5 epsilon.

In a particular embodiment, the T cell activating bispecific antigen binding molecule of the invention comprises

(a) a first Fab molecule which specifically binds to a first antigen;

(b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable
10 domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;

wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen;

wherein the first Fab molecule under (a) comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the
15 HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a
20 light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain
25 variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

According to a further aspect of the invention, the ratio of a desired bispecific antibody compared to undesired side products, in particular Bence Jones-type side products occurring in bispecific antibodies with a VH/VL domain exchange in one of their binding arms, can be
30 improved by the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH1 and CL domains (sometimes referred to herein as “charge modifications”).

Thus, in some embodiments the first antigen binding moiety under (a) is a first Fab molecule which specifically binds to a first antigen, the second antigen binding moiety under (b) is a second Fab molecule which specifically binds to a second antigen wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;

5 and

- i) in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 or the amino acid at position 213 is substituted
10 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 Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 or the amino acid at position 213 is substituted
15 independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one such embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H)
20 (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule 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).

In a further embodiment, in the constant domain CL of the first Fab molecule under a) the amino
25 acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In yet another embodiment, in the constant domain CL of the first Fab molecule under a) the
30 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 the amino acid at position 123 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 in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In another particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In an alternative embodiment, in the constant domain CL of the second Fab molecule 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 in the constant domain CH1 of the second Fab molecule 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).

In a further embodiment, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In still another embodiment, in the constant domain CL of the second Fab molecule 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 the amino acid at position 123 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 in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In one embodiment, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In another embodiment, in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In a particular embodiment, the T cell activating bispecific antigen binding molecule of the invention comprises

- (a) a first Fab molecule which specifically binds to a first antigen;
- (b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other; wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen; wherein the first Fab molecule under (a) comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region,

comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42; and

5 wherein in the constant domain CL of the first Fab molecule 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 the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by
10 lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

15 In some embodiments, the T cell activating bispecific antigen binding molecule according to the invention further comprises a third antigen binding moiety which specifically binds to the first antigen. In particular embodiments, the third antigen binding moiety is identical to the first antigen binding moiety. In one embodiment, the third antigen binding moiety is a Fab molecule. In particular embodiments, the third and the first antigen binding moiety are each a Fab molecule
20 and the third Fab molecule is identical to the first Fab molecule. In these embodiments, the third Fab molecule thus comprises the same amino acid substitutions, if any, as the first Fab molecule. Like the first Fab molecule, the third Fab molecule particularly is a conventional Fab molecule. If a third antigen binding moiety is present, in a particular embodiment the first and the third antigen moiety specifically bind to Tim-3, and the second antigen binding moiety specifically
25 binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon.

In some embodiments of the T cell activating bispecific antigen binding molecule according to the invention the first antigen binding moiety under a) and the second antigen binding moiety under b) are fused to each other, optionally via a peptide linker. In particular embodiments, the first and the second antigen binding moiety are each a Fab molecule. In a specific such
30 embodiment, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. In an alternative such embodiment, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In embodiments wherein either (i) the second Fab

molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule or (ii) the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, additionally the Fab light chain of the Fab molecule and the Fab light chain of the second Fab molecule may
5 be fused to each other, optionally via a peptide linker.

In particular embodiments, the T cell activating bispecific antigen binding molecule according to the invention additionally comprises an Fc domain composed of a first and a second subunit capable of stable association.

The T cell activating bispecific antigen binding molecule according to the invention can have
10 different configurations, i.e. the first, second (and optionally third) antigen binding moiety may be fused to each other and to the Fc domain in different ways. The components may be fused to each other directly or, preferably, via one or more suitable peptide linkers. Where fusion of a Fab molecule is to the N-terminus of a subunit of the Fc domain, it is typically via an immunoglobulin hinge region.

15 In one embodiment, the first and the second antigen binding moiety are each a Fab molecule and the second antigen binding moiety 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 such embodiment, the first antigen binding moiety may be 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 to the N-terminus of the other
20 one of the subunits of the Fc domain.

In one embodiment, the first and the second antigen binding moiety are each a Fab molecule and the first and the second 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. In this embodiment, the T cell activating bispecific antigen binding molecule essentially comprises an immunoglobulin
25 molecule, wherein in one of the Fab arms the heavy and light chain variable regions VH and VL (or the constant regions CH1 and CL in embodiments wherein no charge modifications as described herein are introduced in CH1 and CL domains) are exchanged/replaced by each other (see Figure 1A, D).

In alternative embodiments, a third antigen binding moiety, particularly a third Fab molecule, is
30 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 such embodiment, 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 Fab molecule. In this embodiment, the T cell activating bispecific antigen binding molecule essentially comprises an immunoglobulin molecule, wherein in one of the Fab arms the heavy and light chain variable regions VH and VL (or the constant regions CH1 and CL in embodiments wherein no charge modifications as described herein are introduced in CH1 and CL domains) are exchanged/replaced by each other, and wherein an additional (conventional) Fab molecule is N-terminally fused to said Fab arm (see Figure 1B, E). In another such embodiment, 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 this embodiment, the T cell activating bispecific antigen binding molecule essentially comprises an immunoglobulin molecule with an additional Fab molecule N-terminally fused to one of the immunoglobulin Fab arms, wherein in said additional Fab molecule the heavy and light chain variable regions VH and VL (or the constant regions CH1 and CL in embodiments wherein no charge modifications as described herein are introduced in CH1 and CL domains) are exchanged/replaced by each other (see Figure 1C, F).

In a particular embodiment, the immunoglobulin molecule comprised in the T cell activating bispecific antigen binding molecule according to the invention is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG₁ subclass immunoglobulin. In another embodiment, the immunoglobulin is an IgG₄ subclass immunoglobulin.

In a particular embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;
 - b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;
 - c) a third Fab molecule which specifically binds to the first antigen; and
 - d) an Fc domain composed of a first and a second subunit capable of stable association;
- wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;
- wherein the third Fab molecule under c) is identical to the first Fab molecule under a);
- wherein

(i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) 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 under d), or

5 (ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) and the third Fab molecule under c) 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 under d); and

wherein the first Fab molecule under a) and the third Fab molecule under c) comprise (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the
 10 HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain
 15 variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO:
 20 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

In another embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

25 a) a first Fab molecule which specifically binds to a first antigen;
 b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;
 c) an Fc domain composed of a first and a second subunit capable of stable association;
 30 wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;
 wherein

(i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or

- 5 (ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c); and

wherein the first Fab molecule under a) comprises (i) a heavy chain variable region, particularly
 10 a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1
 15 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3
 20 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

In a further embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

- 25 a) a first Fab molecule which specifically binds to a first antigen;
 b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other; and
 c) an Fc domain composed of a first and a second subunit capable of stable association;
 30 wherein
 (i) the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon; or

(ii) the second antigen is Tim-3 and the first antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

wherein the first Fab molecule under a) and the second Fab molecule under b) 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 under c); and

wherein the Fab molecule which specifically binds to Tim-3 comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-

L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19,

(ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18

and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

In all of the different configurations of the T cell activating bispecific antigen binding molecule according to the invention, the amino acid substitutions described herein, if present, may either be in the CH1 and CL domains of the first and (if present) the third Fab molecule, or in the CH1 and CL domains of the second Fab molecule. Preferably, they are in the CH1 and CL domains of the first and (if present) the third Fab molecule. In accordance with the concept of the invention,

if amino acid substitutions as described herein are made in the first (and, if present, the third) Fab molecule, no such amino acid substitutions are made in the second Fab molecule. Conversely, if amino acid substitutions as described herein are made in the second Fab molecule, no such amino acid substitutions are made in the first (and, if present, the third) Fab molecule. No amino acid substitutions are made in T cell activating bispecific antigen binding molecules comprising a Fab molecule wherein the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other.

In particular embodiments of the T cell activating bispecific antigen binding molecule according to the invention, particularly wherein amino acid substitutions as described herein are made in

the first (and, if present, the third) Fab molecule, the constant domain CL of the first (and, if present, the third) Fab molecule is of kappa isotype. In other embodiments of the T cell activating bispecific antigen binding molecule according to the invention, particularly wherein amino acid substitutions as described herein are made in the second Fab molecule, the constant domain CL of the second Fab molecule is of kappa isotype. In some embodiments, the constant domain CL of the first (and, if present, the third) Fab molecule and the constant domain CL of the second Fab molecule are of kappa isotype.

In a particular embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

- 10 a) a first Fab molecule which specifically binds to a first antigen;
- b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
- c) a third Fab molecule which specifically binds to the first antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association;
- 15 wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;
- wherein the third Fab molecule under c) is identical to the first Fab molecule under a);
- wherein in the constant domain CL of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to
- 20 Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);
- 25 wherein
- (i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) 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 under d), or
- 30 (ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule under a) and the third Fab molecule under c) 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 under d); and

wherein the first Fab molecule under a) and the third Fab molecule under c) comprise (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

15 In an even more particular embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;
- b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;
- 20 c) a third Fab molecule which specifically binds to the first antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association;

wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

wherein the third Fab molecule under c) is identical to the first Fab molecule under a);

25 wherein in the constant domain CL of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);

30 wherein the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab

molecule under b) and the third Fab molecule under c) 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 under d); and

wherein the first Fab molecule under a) and the third Fab molecule under c) comprise (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the
 5 HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15
 10 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain
 15 variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

In another embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

a) a first Fab molecule which specifically binds to a first antigen;

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

c) an Fc domain composed of a first and a second subunit capable of stable association;

wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

25 wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at
 30 position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);

wherein

(i) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab

molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c), or

(ii) the second Fab molecule under b) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule under a), and the first Fab molecule
5 under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of one of the subunits of the Fc domain under c); and

wherein the first Fab molecule under a) comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable
10 region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the
15 HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41
20 and the HVR-L 3 of SEQ ID NO: 42.

In a further embodiment, the invention provides a T cell activating bispecific antigen binding molecule comprising

a) a first Fab molecule which specifically binds to a first antigen;

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

c) an Fc domain composed of a first and a second subunit capable of stable association; wherein

(i) the first antigen is Tim-3 and the second antigen is an activating T cell antigen, particularly
30 CD3, more particularly CD3 epsilon; or

(ii) the second antigen is Tim-3 and the first antigen is an activating T cell antigen, particularly CD3, more particularly CD3 epsilon;

wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index);

wherein the first Fab molecule under a) and the second Fab molecule under b) 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 under c); and

wherein the Fab molecule which specifically binds to Tim-3 comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

In particular embodiments of the T cell activating bispecific antigen binding molecule, the Fc domain is an IgG Fc domain. In a specific embodiment, the Fc domain is an IgG₁ Fc domain. In another specific embodiment, the Fc domain is an IgG₄ Fc domain. In an even more specific embodiment, the Fc domain is an IgG₄ Fc domain comprising the amino acid substitution S228P (Kabat numbering). In particular embodiments the Fc domain is a human Fc domain.

In particular embodiments, the Fc domain comprises a modification promoting the association of the first and the 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.

5 In a particular embodiment the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain. In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In one embodiment, the Fc domain comprises one or more amino acid substitution that reduces binding
10 to an Fc receptor and/or effector function. In one embodiment, the one or more amino acid substitution in the Fc domain that reduces binding to an Fc receptor and/or effector function is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering). In particular embodiments, each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an Fc receptor and/or effector function wherein said
15 amino acid substitutions are L234A, L235A and P329G (Kabat EU index numbering). In one such embodiment, the Fc domain is an IgG₁ Fc domain, particularly a human IgG₁ Fc domain. In other embodiments, each subunit of the Fc domain comprises two amino acid substitutions that reduce binding to an Fc receptor and/or effector function wherein said amino acid substitutions are L235E and P329G (Kabat EU index numbering). In one such embodiment, the Fc domain is
20 an IgG₄ Fc domain, particularly a human IgG₄ Fc domain. In one embodiment, the Fc domain of the T cell activating bispecific antigen binding molecule is an IgG₄ Fc domain and comprises the amino acid substitutions L235E and S228P (SPLE) (Kabat EU index numbering).

In one embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment, the Fc receptor is an activating Fc receptor. In a specific
25 embodiment, the Fc receptor is human FcγRIIa, FcγRI, and/or FcγRIIIa. In one embodiment, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).

In a specific embodiment of the T cell activating bispecific antigen binding molecule according to the invention, the antigen binding moiety which specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon, comprises a heavy chain variable
30 region comprising the HVR-H 1 of SEQ ID NO: 4, the HVR-H 2 of SEQ ID NO: 5, the HVR-H 3 of SEQ ID NO: 6, and a light chain variable region comprising the HVR-L 1 of SEQ ID NO: 8, the HVR-L 2 of SEQ ID NO: 9 and the HVR-L 3 of SEQ ID NO: 10. In an even more specific embodiment, the antigen binding moiety which specifically binds to an activating T cell antigen,

particularly CD3, more particularly CD3 epsilon, comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 7. In some embodiments, the antigen binding moiety which specifically binds to an activating T cell antigen is a Fab molecule. In one specific embodiment, the second antigen binding moiety, particularly Fab molecule, comprised in the T cell activating bispecific antigen binding molecule according to the invention specifically binds to CD3, more particularly CD3 epsilon, and comprises the HVR-H 1 of SEQ ID NO: 4, the HVR-H 2 of SEQ ID NO: 5, the HVR-H 3 of SEQ ID NO: 6, the HVR-L 1 of SEQ ID NO: 8, the HVR-L 2 of SEQ ID NO: 9 and the HVR-L 3 of SEQ ID NO: 10. In an even more specific embodiment, said second antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7.

15 In a further specific embodiment of the T cell activating bispecific antigen binding molecule according to the invention, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, the HVR-H 3 of SEQ ID NO: 16, the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19. In a further specific embodiment of the T cell activating bispecific antigen binding molecule according to the invention, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, the HVR-H 3 of SEQ ID NO: 16, the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19. In a further specific embodiment of the T cell activating bispecific antigen binding molecule according to the invention, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38, the HVR-H 3 of SEQ ID NO: 39, the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42. In an even more specific embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 20 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 21. In a

further specific embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 34. In yet another specific embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36. In still a further specific embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 42 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 44. In another specific embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46.

In one specific embodiment, the first (and, if present, the third) antigen binding moiety, particularly Fab molecule, comprised in the T cell activating bispecific antigen binding molecule according to the invention specifically binds to Tim-3, and comprises the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, the HVR-H 3 of SEQ ID NO: 16, the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19. In an even more specific embodiment, said first (and, if present, said third) antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 20 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 21. In a particular specific embodiment, the first (and, if present, the third) antigen binding moiety, particularly Fab molecule, comprised in the T cell activating bispecific antigen binding molecule according to the invention specifically binds to Tim-3, and

comprises the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, the HVR-H 3 of SEQ ID NO: 16, the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19. In an even more specific embodiment, said first (and, if present, said third) antigen binding moiety, particularly Fab molecule, comprises (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 34, or (ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 36. In another specific embodiment, the first (and, if present, the third) antigen binding moiety, particularly Fab molecule, comprised in the T cell activating bispecific antigen binding molecule according to the invention specifically binds to Tim-3, and comprises the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38, the HVR-H 3 of SEQ ID NO: 39, the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42. In an even more specific embodiment, said first (and, if present, said third) antigen binding moiety, particularly Fab molecule, comprises (i) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 43 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 44, or (ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 46.

In a particular aspect, the invention provides a T cell activating bispecific antigen binding molecule comprising

- a) a first Fab molecule which specifically binds to a first antigen;
- b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other;
- c) a third Fab molecule which specifically binds to the first antigen; and
- d) an Fc domain composed of a first and a second subunit capable of stable association;

wherein

- (i) the first antigen is Tim-3 and the second antigen is CD3, particularly CD3 epsilon;
- (ii) the first Fab molecule under a) and the third Fab molecule under c) each comprise (i) the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, the HVR-H 3 of SEQ ID NO: 16, the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, the HVR-H 3 of

SEQ ID NO: 16, the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38, the HVR-H 3 of SEQ ID NO: 39, the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42, and the second Fab molecule under b) comprises the HVR-H 1 of SEQ ID NO: 4, the HVR-H 2 of SEQ ID NO: 5, the HVR-H 3 of SEQ ID NO: 6, the HVR-L 1 of SEQ ID NO: 8, the HVR-L 2 of SEQ ID NO: 9 and the HVR-L 3 of SEQ ID NO: 10; and

(iii) the first Fab molecule under a) is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule under b), and the second Fab molecule under b) and the third Fab molecule under c) 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 under d).

In one embodiment, in the second Fab molecule under b) the variable domains VL and VH are replaced by each other and further (iv) in the constant domain CL of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R), particularly by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) and the third Fab molecule under c) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

According to another aspect of the invention there is provided one or more isolated polynucleotide(s) encoding a T cell activating bispecific antigen binding molecule of the invention. The invention further provides one or more expression vector(s) comprising the isolated polynucleotide(s) of the invention, and a host cell comprising the isolated polynucleotide(s) or the expression vector(s) of the invention. In some embodiments the host cell is a eukaryotic cell, particularly a mammalian cell.

In another aspect is provided a method of producing the T cell activating bispecific antigen binding molecule of the invention, comprising the steps of a) culturing the host cell of the invention 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 also encompasses a T cell activating bispecific antigen binding molecule produced by the method of the invention.

The invention further provides a pharmaceutical composition comprising the T cell activating bispecific antigen binding molecule of the invention and a pharmaceutically acceptable carrier.

Also encompassed by the invention are methods of using the T cell activating bispecific antigen binding molecule and pharmaceutical composition of the invention. In one aspect the invention provides a T cell activating bispecific antigen binding molecule or a pharmaceutical composition of the invention for use as a medicament. In one aspect is provided a T cell activating bispecific antigen binding molecule or a pharmaceutical composition 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 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 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.

The invention 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 in the presence of a T cell, particularly a cytotoxic T cell.

Brief Description of the Drawings

FIGURE 1. Exemplary configurations of the T cell activating bispecific antigen binding molecules (TCBs) of the invention. (A, D) Illustration of the “1+1 CrossMab” molecule. (B, E) Illustration of the “2+1 IgG Crossfab” molecule with alternative order of Crossfab and Fab components (“inverted”). (C, F) Illustration of the “2+1 IgG Crossfab” molecule. (G, K) Illustration of the “1+1 IgG Crossfab” molecule with alternative order of Crossfab and Fab components (“inverted”). (H, L) Illustration of the “1+1 IgG Crossfab” molecule. (I, M) Illustration of the “2+1 IgG Crossfab” molecule with two CrossFabs. (J, N) Illustration of the “2+1 IgG Crossfab” molecule with two CrossFabs and alternative order of Crossfab and Fab components (“inverted”). (O, S) Illustration of the “Fab-Crossfab” molecule. (P, T) Illustration of the “Crossfab-Fab” molecule. (Q, U) Illustration of the “(Fab)₂-Crossfab” molecule. (R, V) Illustration of the “Crossfab-(Fab)₂” molecule. (W, Y) Illustration of the “Fab-(Crossfab)₂”

molecule. (X, Z) Illustration of the “(Crossfab)₂-Fab” molecule. Black dot: optional modification in the Fc domain promoting heterodimerization. ++, --: amino acids of opposite charges optionally introduced in the CH1 and CL domains. Crossfab molecules are depicted as comprising an exchange of VH and VL regions, but may – in embodiments wherein no charge modifications are introduced in CH1 and CL domains – alternatively comprise an exchange of the CH1 and CL domains.

FIGURE 2. Illustration of the TCB molecule prepared in Example 1: “2+1 IgG CrossFab, inverted” with charge modifications (VH/VL exchange in CD3 binder, charge modification in STEAP-1 binders, EE = 147E, 213E; RK = 123R, 124K).

FIGURE 3. CE-SDS analysis of the TCB molecule prepared in Example 1 (final purified preparations, electropherogram, lane A = non-reduced, lane B = reduced).

FIGURE 4. Expression level of TIM-3 on different hematological tumor cell lines by FACS. (A) Pfeiffer (NHBCL); (B) RPMI-8226 (MM); (C) SR (LCIBL); (D) Karpas-299 (NHTCL); (E) HDLM-2 (HL); (F) CMK (AML); (G) KG-1 (AML); (H) TF-1 (AML); (I) Kasumi-1 (AML); (J) Raji (BL).

FIGURE 5. Dose dependent tumor cell lysis of lymphoid und myeloid tumor cell lines expressing different levels of TIM3 with TIM3 TCB molecule as measured by LDH release assay. (A-B) CHO K1 cells stably expressing human TIM-3 (A, EC50 3 pM), Pfeiffer cells (B, EC50 60 pM), 24 h LDH release, effectors = thawed expanded T cells, E:T 1:1. (C-F) Pfeiffer cells (C), RPMI-8226 cells (D), Karpas-299 cells (E), CMK cells (F), 66 h LDH release, effectors = expanded T cells, E:T 3:1.

Detailed Description of the Invention

Definitions

Terms are used herein as generally used in the art, unless otherwise defined in the following.

As used herein, the term "antigen binding molecule" refers in its broadest sense to a molecule that specifically binds an antigenic determinant. Examples of antigen binding molecules are immunoglobulins and derivatives, e.g. fragments, thereof.

The term “bispecific” means that the antigen binding molecule is able to specifically bind to at least two distinct antigenic determinants. Typically, a bispecific antigen binding molecule comprises two antigen binding sites, each of which is specific for a different antigenic determinant. In certain embodiments the bispecific antigen binding molecule is capable of

simultaneously binding two antigenic determinants, particularly two antigenic determinants expressed on two distinct cells.

The term "valent" as used herein denotes the presence of a specified number of antigen binding sites in an antigen binding molecule. As such, the term "monovalent binding to an antigen" denotes the presence of one (and not more than one) antigen binding site specific for the antigen in the antigen binding molecule.

An "antigen binding site" refers to the site, i.e. one or more amino acid residues, of an antigen binding molecule which provides interaction with the antigen. For example, the antigen binding site of an antibody comprises amino acid residues from the complementarity determining regions (CDRs). A native immunoglobulin molecule typically has two antigen binding sites, a Fab molecule typically has a single antigen binding site.

As used herein, the term "antigen binding moiety" refers to a polypeptide molecule that specifically binds to an antigenic determinant. In one embodiment, an antigen binding moiety is able to direct the entity to which it is attached (e.g. a second antigen binding moiety) to a target site, for example to a specific type of tumor cell or tumor stroma bearing the antigenic determinant. In another embodiment an antigen binding moiety is able to activate signaling through its target antigen, for example a T cell receptor complex antigen. Antigen binding moieties include antibodies and fragments thereof as further defined herein. Particular antigen binding moieties include an antigen binding domain of an antibody, comprising an antibody heavy chain variable region and an antibody light chain variable region. In certain embodiments, the antigen binding moieties may comprise antibody constant regions as further defined herein and known in the art. Useful heavy chain constant regions include any of the five isotypes: α , δ , ϵ , γ , or μ . Useful light chain constant regions include any of the two isotypes: κ and λ .

As used herein, the term "antigenic determinant" is synonymous with "antigen" and "epitope," and refers to a site (e.g. a contiguous stretch of amino acids or a conformational configuration made up of different regions of non-contiguous amino acids) on a polypeptide macromolecule to which an antigen binding moiety binds, forming an antigen binding moiety-antigen complex. Useful antigenic determinants can be found, for example, on the surfaces of tumor cells, on the surfaces of virus-infected cells, on the surfaces of other diseased cells, on the surface of immune cells, free in blood serum, and/or in the extracellular matrix (ECM). The proteins referred to as antigens herein (e.g. CD3) can be any native form the proteins from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g. mice and rats), unless otherwise indicated. In a particular embodiment the antigen is a human protein. Where reference

is made to a specific protein herein, the term encompasses the “full-length”, unprocessed protein as well as any form of the protein that results from processing in the cell. The term also encompasses naturally occurring variants of the protein, e.g. splice variants or allelic variants. An exemplary human protein useful as antigen is CD3, particularly the epsilon subunit of CD3 (see UniProt no. P07766 (version 130), NCBI RefSeq no. NP_000724.1, SEQ ID NO: 1 for the human sequence; or UniProt no. Q95LI5 (version 49), NCBI GenBank no. BAB71849.1, SEQ ID NO: 2 for the cynomolgus [*Macaca fascicularis*] sequence), or Tim-3 (see UniProt no. Q8TDQ0). Tim-3, also known as “T cell Immunoglobulin- and Mucin domain-containing molecule 3” or Hepatitis A virus cellular receptor 2 (HAVCR2), refers to a T helper cell type 1-specific cell surface protein that regulates macrophage activation and the severity of inflammatory conditions. The nucleotide and protein sequences of TIM3 are known for many species. For example, the human amino acid sequence can be found under Uniprot accession number Q8TDQ0 or NCBI RefSeq no. NP_116171.3. The human protein is characterized by an extracellular domain comprising an Ig like domain and a mucin domain (further comprising O-linked and N-linked glycosylation sites) comprising approximately amino acids 22-202, a transmembrane domain (amino acids 203-223), and an intracellular (cytoplasmic) domain (amino acids 224-301). The term “Tim-3” includes variants, isoforms, species homologs of human Tim-3, and analogs having at least one common epitope with Tim-3.. In certain embodiments the T cell activating bispecific antigen binding molecule of the invention binds to an epitope of CD3 or Tim-3 that is conserved among the CD3 or Tim-3 antigens from different species.

By “specific binding” is meant that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen binding moiety to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)). In one embodiment, the extent of binding of an antigen binding moiety to an unrelated protein is less than about 10% of the binding of the antigen binding moiety to the antigen as measured, e.g., by SPR. In certain embodiments, an antigen binding moiety that binds to the antigen, or an antigen binding molecule comprising that antigen binding moiety, has a dissociation constant (K_D) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M , e.g., from 10^{-9} M to 10^{-13} M).

“Affinity” refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (e.g., a receptor) and its binding partner (e.g., a ligand). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., an antigen binding moiety and an antigen, or a receptor and its ligand). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D), which is the ratio of dissociation and association rate constants (k_{off} and k_{on} , respectively). Thus, equivalent affinities may comprise different rate constants, as long as the ratio of the rate constants remains the same. Affinity can be measured by well established methods known in the art, including those described herein. A particular method for measuring affinity is Surface Plasmon Resonance (SPR).

“Reduced binding”, for example reduced binding to an Fc receptor, refers to a decrease in affinity for the respective interaction, as measured for example by SPR. For clarity the term includes also reduction of the affinity to zero (or below the detection limit of the analytic method), i.e. complete abolishment of the interaction. Conversely, “increased binding” refers to an increase in binding affinity for the respective interaction.

An “activating T cell antigen” as used herein refers to an antigenic determinant expressed on the surface of a T lymphocyte, particularly a cytotoxic T lymphocyte, which is capable of inducing T cell activation upon interaction with an antigen binding molecule. Specifically, interaction of an antigen binding molecule with an activating T cell antigen may induce T cell activation by triggering the signaling cascade of the T cell receptor complex. In a particular embodiment the activating T cell antigen is CD3, particularly the epsilon subunit of CD3 (see UniProt no. P07766 (version 130), NCBI RefSeq no. NP_000724.1, SEQ ID NO: 1 for the human sequence; or UniProt no. Q95LI5 (version 49), NCBI GenBank no. BAB71849.1, SEQ ID NO: 2 for the cynomolgus [*Macaca fascicularis*] sequence).

“T cell activation” as used herein refers to one or more cellular response of a T lymphocyte, particularly a cytotoxic T lymphocyte, selected from: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers. The T cell activating bispecific antigen binding molecules of the invention are capable of inducing T cell activation. Suitable assays to measure T cell activation are known in the art described herein.

A “target cell antigen” as used herein refers to an antigenic determinant presented on the surface of a target cell, for example a cell in a tumor such as a cancer cell or a cell of the tumor stroma. In a particular embodiment, the target cell antigen is Tim-3, particularly human Tim-3.

As used herein, the terms “first”, “second” or “third” with respect to Fab molecules etc., are used for convenience of distinguishing when there is more than one of each type of moiety. Use of these terms is not intended to confer a specific order or orientation of the T cell activating bispecific antigen binding molecule unless explicitly so stated.

5 A “Fab molecule” refers to a protein consisting of the VH and CH1 domain of the heavy chain (the “Fab heavy chain”) and the VL and CL domain of the light chain (the “Fab light chain”) of an immunoglobulin.

By “fused” is meant that the components (e.g. a Fab molecule and an Fc domain subunit) are linked by peptide bonds, either directly or via one or more peptide linkers.

10 As used herein, the term “single-chain” refers to a molecule comprising amino acid monomers linearly linked by peptide bonds. In certain embodiments, one of the antigen binding moieties is a single-chain Fab molecule, i.e. a Fab molecule wherein the Fab light chain and the Fab heavy chain are connected by a peptide linker to form a single peptide chain. In a particular such embodiment, the C-terminus of the Fab light chain is connected to the N-terminus of the Fab
15 heavy chain in the single-chain Fab molecule.

By a “crossover” Fab molecule (also termed “Crossfab”) is meant a Fab molecule wherein the variable domains or the constant domains of the Fab heavy and light chain are exchanged (i.e. replaced by each other), i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable domain VL and the heavy chain constant domain 1 CH1 (VL-CH1, in N-
20 to C-terminal direction), and a peptide chain composed of the heavy chain variable domain VH and the light chain constant domain CL (VH-CL, in N- to C-terminal direction). For clarity, in a crossover Fab molecule wherein the variable domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain constant domain 1 CH1 is referred to herein as the “heavy chain” of the (crossover) Fab molecule. Conversely, in a
25 crossover Fab molecule wherein the constant domains of the Fab light chain and the Fab heavy chain are exchanged, the peptide chain comprising the heavy chain variable domain VH is referred to herein as the “heavy chain” of the (crossover) Fab molecule.

In contrast thereto, by a “conventional” Fab molecule is meant a Fab molecule in its natural format, i.e. comprising a heavy chain composed of the heavy chain variable and constant
30 domains (VH-CH1, in N- to C-terminal direction), and a light chain composed of the light chain variable and constant domains (VL-CL, in N- to C-terminal direction).

The term “immunoglobulin molecule” refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric

glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable region, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a light chain variable region, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called α (IgA), δ (IgD), ϵ (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. γ_1 (IgG₁), γ_2 (IgG₂), γ_3 (IgG₃), γ_4 (IgG₄), α_1 (IgA₁) and α_2 (IgA₂). The light chain of an immunoglobulin may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, and antibody fragments so long as they exhibit the desired antigen-binding activity.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabodies, linear antibodies, single-chain antibody molecules (e.g. scFv), and single-domain antibodies. For a review of certain antibody fragments, see Hudson et al., Nat Med 9, 129-134 (2003). For a review of scFv fragments, see e.g. Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')₂ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat Med 9, 129-134 (2003); and Hollinger et al., Proc Natl Acad Sci USA 90, 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat Med 9, 129-134 (2003). Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see e.g. U.S. Patent No. 6,248,516 B1). Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact

antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

The term "antigen binding domain" refers to the part of an antibody that comprises the area which specifically binds to and is complementary to part or all of an antigen. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Particularly, an antigen binding domain comprises an antibody light chain variable domain (VL) and an antibody heavy chain variable domain (VH).

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). See, e.g., Kindt et al., *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007). A single VH or VL domain may be sufficient to confer antigen-binding specificity.

The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed.

Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

As used herein, the amino acid positions of all constant regions and domains of the heavy and light chain are numbered according to the Kabat numbering system described in Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and is referred to as “numbering according to Kabat” or “Kabat numbering” herein. Specifically the Kabat numbering system (see pages 647-660 of Kabat, et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991)) is used for the light chain constant domain CL of kappa and lambda isotype and the Kabat EU index numbering system (see pages 661-723) is used for the heavy chain constant domains (CH1, Hinge, CH2 and CH3), which is herein further clarified by referring to “numbering according to Kabat EU index” in this case.

The polypeptide sequences of the sequence listing are not numbered according to the Kabat numbering system. However, it is well within the ordinary skill of one in the art to convert the numbering of the sequences of the Sequence Listing to Kabat numbering.

“Framework” or “FR” refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

A “humanized” antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. Such variable domains are referred to herein as “humanized variable region”. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization. Other forms of “humanized antibodies” encompassed by the present invention are those in which the constant region has been additionally modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding.

The “class” of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁,

IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

The term “Fc domain” or “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term
5 includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, antibodies produced by host cells may undergo post-translational cleavage of one or more, particularly one or two, amino acids from the C-terminus of the heavy chain.

10 Therefore an antibody produced by a host cell by expression of a specific nucleic acid molecule encoding a full-length heavy chain may include the full-length heavy chain, or it may include a cleaved variant of the full-length heavy chain (also referred to herein as a “cleaved variant heavy chain”). This may be the case where the final two C-terminal amino acids of the heavy chain are glycine (G446) and lysine (K447, numbering according to Kabat EU index). Therefore, the C-
15 terminal lysine (Lys447), or the C-terminal glycine (Gly446) and lysine (K447), of the Fc region may or may not be present. Amino acid sequences of heavy chains including Fc domains (or a subunit of an Fc domain as defined herein) are denoted herein without C-terminal glycine-lysine dipeptide if not indicated otherwise. In one embodiment of the invention, a heavy chain including a subunit of an Fc domain as specified herein, comprised in a T cell activating
20 bispecific antigen binding molecule according to the invention, comprises an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat).

In one embodiment of the invention, a heavy chain including a subunit of an Fc domain as specified herein, comprised in a T cell activating bispecific antigen binding molecule according to the invention, comprises an additional C-terminal glycine residue (G446, numbering
25 according to EU index of Kabat). Compositions of the invention, such as the pharmaceutical compositions described herein, comprise a population of T cell activating bispecific antigen binding molecules of the invention. The population of T cell activating bispecific antigen binding molecule may comprise molecules having a full-length heavy chain and molecules having a cleaved variant heavy chain. The population of T cell activating bispecific antigen
30 binding molecules may consist of a mixture of molecules having a full-length heavy chain and molecules having a cleaved variant heavy chain, wherein at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the T cell activating bispecific antigen binding molecules have a cleaved variant heavy chain. In one embodiment of the invention a composition comprising a

population of T cell activating bispecific antigen binding molecules of the invention comprises an T cell activating bispecific antigen binding molecule comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). In one embodiment of the invention a composition comprising a population of T cell activating bispecific antigen binding molecules of the invention comprises an T cell activating bispecific antigen binding molecule comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat). In one embodiment of the invention such a composition comprises a population of T cell activating bispecific antigen binding molecules comprised of molecules comprising a heavy chain including a subunit of an Fc domain as specified herein; molecules comprising a heavy chain including a subunit of a Fc domain as specified herein with an additional C-terminal glycine residue (G446, numbering according to EU index of Kabat); and molecules comprising a heavy chain including a subunit of an Fc domain as specified herein with an additional C-terminal glycine-lysine dipeptide (G446 and K447, numbering according to EU index of Kabat). Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991 (see also above). A “subunit” of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

A “modification promoting the association of the first and the second subunit of the Fc domain” is a manipulation of the peptide backbone or the post-translational modifications of an Fc domain subunit that reduces or prevents the association of a polypeptide comprising the Fc domain subunit with an identical polypeptide to form a homodimer. A modification promoting association as used herein particularly includes separate modifications made to each of the two Fc domain subunits desired to associate (i.e. the first and the second subunit of the Fc domain), wherein the modifications are complementary to each other so as to promote association of the two Fc domain subunits. For example, a modification promoting association may alter the structure or charge of one or both of the Fc domain subunits so as to make their association sterically or electrostatically favorable, respectively. Thus, (hetero)dimerization occurs between

a polypeptide comprising the first Fc domain subunit and a polypeptide comprising the second Fc domain subunit, which might be non-identical in the sense that further components fused to each of the subunits (e.g. antigen binding moieties) are not the same. In some embodiments the modification promoting association comprises an amino acid mutation in the Fc domain, specifically an amino acid substitution. In a particular embodiment, the modification promoting association comprises a separate amino acid mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

The term “effector functions” refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), cytokine secretion, immune complex-mediated antigen uptake by antigen presenting cells, down regulation of cell surface receptors (e.g. B cell receptor), and B cell activation.

As used herein, the terms “engineer, engineered, engineering”, are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of the amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches.

The term “amino acid mutation” as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., reduced binding to an Fc receptor, or increased association with another peptide. Amino acid sequence deletions and insertions include amino- and/or carboxy-terminal deletions and insertions of amino acids. Particular amino acid mutations are amino acid substitutions. For the purpose of altering e.g. the binding characteristics of an Fc region, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties, are particularly preferred. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids (e.g. 4-hydroxyproline, 3-methylhistidine, ornithine, homoserine, 5-hydroxylysine). Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is

contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid mutation. For example, a substitution from proline at position 329 of the Fc domain to glycine can be indicated as 329G, 5 G329, G₃₂₉, P329G, or Pro329Gly.

As used herein, term "polypeptide" refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other 10 term used to refer to a chain of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic 15 cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis. A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 20 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded.

By an "isolated" polypeptide or a variant, or derivative thereof is intended a polypeptide that is 25 not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for the purpose of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or 30 partially or substantially purified by any suitable technique.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and

introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "polynucleotide" refers to an isolated nucleic acid molecule or construct, e.g. messenger RNA (mRNA), virally-derived RNA, or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (e.g. an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid molecule" refers to

any one or more nucleic acid segments, e.g. DNA or RNA fragments, present in a polynucleotide.

By "isolated" nucleic acid molecule or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a polypeptide contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the present invention, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g. ALIGN-2).

The term "expression cassette" refers to a polynucleotide generated recombinantly or synthetically, with a series of specified nucleic acid elements that permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment.

5 Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid sequence to be transcribed and a promoter. In certain embodiments, the expression cassette of the invention comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

The term "vector" or "expression vector" is synonymous with "expression construct" and refers
10 to a DNA molecule that is used to introduce and direct the expression of a specific gene to which it is operably associated in a target cell. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. The expression vector of the present invention comprises an expression cassette. Expression vectors allow transcription of large amounts of stable mRNA. Once the
15 expression vector is inside the target cell, the ribonucleic acid molecule or protein that is encoded by the gene is produced by the cellular transcription and/or translation machinery. In one embodiment, the expression vector of the invention comprises an expression cassette that comprises polynucleotide sequences that encode bispecific antigen binding molecules of the invention or fragments thereof.

20 The terms "host cell", "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain
25 mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate the bispecific antigen binding molecules of the present invention. Host cells include cultured cells, *e.g.* mammalian cultured cells, such as CHO cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER
30 cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells, and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

An “activating Fc receptor” is an Fc receptor that following engagement by an Fc domain of an antibody elicits signaling events that stimulate the receptor-bearing cell to perform effector functions. Human activating Fc receptors include Fc γ RIIIa (CD16a), Fc γ RI (CD64), Fc γ RIIa (CD32), and Fc α RI (CD89).

- 5 Antibody-dependent cell-mediated cytotoxicity (ADCC) is an immune mechanism leading to the lysis of antibody-coated target cells by immune effector cells. The target cells are cells to which antibodies or derivatives thereof comprising an Fc region specifically bind, generally via the protein part that is N-terminal to the Fc region. As used herein, the term “reduced ADCC” is defined as either a reduction in the number of target cells that are lysed in a given time, at a
10 given concentration of antibody in the medium surrounding the target cells, by the mechanism of ADCC defined above, and/or an increase in the concentration of antibody in the medium surrounding the target cells, required to achieve the lysis of a given number of target cells in a given time, by the mechanism of ADCC. The reduction in ADCC is relative to the ADCC mediated by the same antibody produced by the same type of host cells, using the same standard
15 production, purification, formulation and storage methods (which are known to those skilled in the art), but that has not been engineered. For example the reduction in ADCC mediated by an antibody comprising in its Fc domain an amino acid substitution that reduces ADCC, is relative to the ADCC mediated by the same antibody without this amino acid substitution in the Fc domain. Suitable assays to measure ADCC are well known in the art (see e.g. PCT publication
20 no. WO 2006/082515 or PCT publication no. WO 2012/130831).

An “effective amount” of an agent refers to the amount that is necessary to result in a physiological change in the cell or tissue to which it is administered.

- A “therapeutically effective amount” of an agent, e.g. a pharmaceutical composition, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic
25 or prophylactic result. A therapeutically effective amount of an agent for example eliminates, decreases, delays, minimizes or prevents adverse effects of a disease.

- An “individual” or “subject” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g. cows, sheep, cats, dogs, and horses), primates (e.g. humans and non-human primates such as monkeys), rabbits, and rodents (e.g. mice and rats). Particularly, the
30 individual or subject is a human.

The term “pharmaceutical composition” refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which

contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A “pharmaceutically acceptable carrier” refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, “treatment” (and grammatical variations thereof such as “treat” or “treating”) refers to clinical intervention in an attempt to alter the natural course of a disease in the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, T cell activating bispecific antigen binding molecules of the invention are used to delay development of a disease or to slow the progression of a disease.

The term “package insert” is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

Detailed Description of the Embodiments

The invention provides a T cell activating bispecific antigen binding molecule with favorable properties for therapeutic application, in particular with respect to efficacy and safety as well as produceability (e.g. with respect to purity, yield).

Charge modifications

The T cell activating bispecific antigen binding molecules of the invention may comprise amino acid substitutions in Fab molecules comprised therein which are particularly efficient in reducing mispairing of light chains with non-matching heavy chains (Bence-Jones-type side products), which can occur in the production of Fab-based bi-/multispecific antigen binding molecules with a VH/VL exchange in one (or more, in case of molecules comprising more than two antigen-binding Fab molecules) of their binding arms (see also PCT application no. PCT/EP2015/057165, particularly the examples therein, incorporated herein by reference in its entirety).

Accordingly, in particular embodiments, the T cell activating bispecific antigen binding molecule of the invention comprises

(a) a first Fab molecule which specifically binds to a first antigen
 (b) a second Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other, wherein the first antigen is an activating T cell antigen and the second antigen is Tim-3, or the first antigen is Tim-3 and the second antigen is an activating T cell antigen; and
 5 wherein

- i) in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at
 10 position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index); or
- ii) in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by a positively charged amino acid (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid
 15 at position 147 or the amino acid at position 213 is substituted by a negatively charged amino acid (numbering according to Kabat EU index).

The T cell activating bispecific antigen binding molecule does not comprise both modifications mentioned under i) and ii). The constant domains CL and CH1 of the second Fab molecule are not replaced by each other (i.e. remain unexchanged).

20 In one embodiment of the T cell activating bispecific antigen binding molecule according to the invention, in the constant domain CL of the first Fab molecule 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 in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147
 25 or the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a further embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under
 30 a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

In a particular embodiment, in the constant domain CL of the first Fab molecule 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 the amino acid at position 123 is substituted independently by lysine (K),
5 arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred embodiment independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according
10 to Kabat EU index).

In a more particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) or arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at
15 position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

In an even more particular embodiment, in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to
20 Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

25 In particular embodiments, the constant domain CL of the first Fab molecule under a) is of kappa isotype.

Alternatively, the amino acid substitutions according to the above embodiments may be made in the constant domain CL and the constant domain CH1 of the second Fab molecule under b) instead of in the constant domain CL and the constant domain CH1 of the first Fab molecule
30 under a). In particular such embodiments, the constant domain CL of the second Fab molecule under b) is of kappa isotype.

The T cell activating bispecific antigen binding molecule according to the invention may further comprise a third Fab molecule which specifically binds to the first antigen. In particular embodiments, said third Fab molecule is identical to the first Fab molecule under a). In these embodiments, the amino acid substitutions according to the above embodiments will be made in the constant domain CL and the constant domain CH1 of each of the first Fab molecule and the third Fab molecule. Alternatively, the amino acid substitutions according to the above embodiments may be made in the constant domain CL and the constant domain CH1 of the second Fab molecule under b), but not in the constant domain CL and the constant domain CH1 of the first Fab molecule and the third Fab molecule.

- 10 In particular embodiments, the T cell activating bispecific antigen binding molecule according to the invention further comprises an Fc domain composed of a first and a second subunit capable of stable association.

T cell activating bispecific antigen binding molecule formats

- 15 The components of the T cell activating bispecific antigen binding molecule can be fused to each other in a variety of configurations. Exemplary configurations are depicted in Figure 1.

- In particular embodiments, the antigen binding moieties comprised in the T cell activating bispecific antigen binding molecule are Fab molecules. In such embodiments, the first, second, third etc. antigen binding moiety may be referred to herein as first, second, third etc. Fab molecule, respectively. Furthermore, in particular embodiments, the T cell activating bispecific antigen binding molecule comprises an Fc domain composed of a first and a second subunit capable of stable association.

In some embodiments, the second Fab 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.

- 25 In one such embodiment, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In a specific such embodiment, the T cell activating bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the second Fab 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. Such a configuration is schematically depicted

in Figures 1G and 1K. Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

In another such embodiment, the first Fab molecule 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 specific such
5 embodiment, the T cell activating bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the first and the second Fab 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. Such a configuration is schematically depicted in Figures 1A and 1D. The first and the
10 second Fab molecule may be fused to the Fc domain directly or through a peptide linker. In a particular embodiment the first and the second Fab molecule are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region, particularly where the Fc domain is an IgG₁ Fc domain.

In other embodiments, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to
15 the N-terminus of the first or second subunit of the Fc domain.

In one such embodiment, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. In a specific such embodiment, the T cell activating bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, the Fc domain composed of a first and a second subunit, and
20 optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the first Fab 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. Such a configuration is schematically depicted in Figures 1H and 1L. Optionally, the Fab light chain of the first Fab
25 molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

The Fab molecules may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers
30 include, for example, (G₄S)_n, (SG₄)_n, (G₄S)_n or G₄(SG₄)_n peptide linkers. "n" is generally an integer from 1 to 10, typically from 2 to 4. In one embodiment said peptide linker has a length of at least 5 amino acids, in one embodiment a length of 5 to 100, in a further embodiment of 10 to 50 amino acids. In one embodiment said peptide linker is (GxS)_n or (GxS)_nG_m with G=glycine,

S=serine, and ($x=3$, $n=3, 4, 5$ or 6 , and $m=0, 1, 2$ or 3) or ($x=4$, $n=2, 3, 4$ or 5 and $m=0, 1, 2$ or 3), in one embodiment $x=4$ and $n=2$ or 3 , in a further embodiment $x=4$ and $n=2$. In one embodiment said peptide linker is $(G_4S)_2$. A particularly suitable peptide linker for fusing the Fab light chains of the first and the second Fab molecule to each other is $(G_4S)_2$. An exemplary peptide linker suitable for connecting the Fab heavy chains of the first and the second Fab fragments comprises the sequence $(D)-(G_4S)_2$ (SEQ ID NOs 11 and 12). Another suitable such linker comprises the sequence $(G_4S)_4$. Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly where a Fab molecule is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

A T cell activating bispecific antigen binding molecule with a single antigen binding moiety (such as a Fab molecule) capable of specific binding to a target cell antigen (for example as shown in Figure 1A, D, G, H, K, L) is useful, particularly in cases where internalization of the target cell antigen is to be expected following binding of a high affinity antigen binding moiety.

In such cases, the presence of more than one antigen binding moiety specific for the target cell antigen may enhance internalization of the target cell antigen, thereby reducing its availability.

In many other cases, however, it will be advantageous to have a T cell activating bispecific antigen binding molecule comprising two or more antigen binding moieties (such as Fab molecules) specific for a target cell antigen (see examples shown in Figure 1B, 1C, 1E, 1F, 1I, 1J, 1M or 1N), for example to optimize targeting to the target site or to allow crosslinking of target cell antigens.

Accordingly, in particular embodiments, the T cell activating bispecific antigen binding molecule of the invention further comprises a third Fab molecule which specifically binds to the first antigen. The first antigen preferably is the target cell antigen, i.e. Tim-3. In one embodiment, the third Fab molecule is a conventional Fab molecule. In one embodiment, the third Fab molecule is identical to the first Fab molecule (i.e. the first and the third Fab molecule comprise the same heavy and light chain amino acid sequences and have the same arrangement of domains (i.e. conventional or crossover)). In a particular embodiment, the second Fab molecule specifically binds to an activating T cell antigen, particularly CD3, and the first and third Fab molecule specifically bind to Tim-3.

In alternative embodiments, the T cell activating bispecific antigen binding molecule of the invention further comprises a third Fab molecule which specifically binds to the second antigen. In these embodiments, the second antigen preferably is the target cell antigen, i.e. Tim-3. In one

such embodiment, the third Fab molecule is a crossover Fab molecule (a Fab molecule wherein the variable domains VH and VL or the constant domains CL and CH1 of the Fab heavy and light chains are exchanged / replaced by each other). In one such embodiment, the third Fab molecule is identical to the second Fab molecule (i.e. the second and the third Fab molecule
5 comprise the same heavy and light chain amino acid sequences and have the same arrangement of domains (i.e. conventional or crossover)). In one such embodiment, the first Fab molecule specifically binds to an activating T cell antigen, particularly CD3, and the second and third Fab molecule specifically bind to Tim-3.

In one embodiment, the third Fab molecule is fused at the C-terminus of the Fab heavy chain to
10 the N-terminus of the first or second subunit of the Fc domain.

In a particular embodiment, the second and the third Fab 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 Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In a specific such embodiment, the T cell
15 activating bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the
20 first subunit of the Fc domain, and wherein the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. Such a configuration is schematically depicted in Figure 1B and 1E (particular embodiments, wherein the third Fab molecule is a conventional Fab molecule and preferably identical to the first Fab molecule), and Figure 1I and 1M (alternative embodiments, wherein the third Fab molecule is a
25 crossover Fab molecule and preferably identical to the second Fab molecule). The second and the third Fab molecule may be fused to the Fc domain directly or through a peptide linker. In a particular embodiment the second and the third Fab molecule are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region, particularly where the Fc domain is an IgG₁ Fc domain.
30 Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

In another embodiment, the first and the third Fab 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

Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. In a specific such embodiment, the T cell activating bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, the Fc domain composed of a first and a second subunit, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first subunit of the Fc domain, and wherein the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the second subunit of the Fc domain. Such a configuration is schematically depicted in Figure 1C and 1F (particular embodiments, wherein the third Fab molecule is a conventional Fab molecule and preferably identical to the first Fab molecule) and in Figure 1J and 1N (alternative embodiments, wherein the third Fab molecule is a crossover Fab molecule and preferably identical to the second Fab molecule). The first and the third Fab molecule may be fused to the Fc domain directly or through a peptide linker. In a particular embodiment the first and the third Fab molecule are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG₁ hinge region, particularly where the Fc domain is an IgG₁ Fc domain. Optionally, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule may additionally be fused to each other.

In configurations of the T cell activating bispecific antigen binding molecule wherein a Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of each of the subunits of the Fc domain through an immunoglobulin hinge regions, the two Fab molecules, the hinge regions and the Fc domain essentially form 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 IgG₁ subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG₄ subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.

In some of the T cell activating bispecific antigen binding molecule of the invention, the Fab light chain of the first Fab molecule and the Fab light chain of the second Fab molecule are fused to each other, optionally via a peptide linker. Depending on the configuration of the first and the second Fab molecule, the Fab light chain of the first Fab molecule may be fused at its C-terminus to the N-terminus of the Fab light chain of the second Fab molecule, or the Fab light

chain of the second Fab molecule may be fused at its C-terminus to the N-terminus of the Fab light chain of the first Fab molecule. Fusion of the Fab light chains of the first and the second Fab molecule further reduces mispairing of unmatched Fab heavy and light chains, and also reduces the number of plasmids needed for expression of some of the T cell activating bispecific antigen binding molecules of the invention.

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit ($VL_{(2)}$ -CH1₍₂₎-CH2-CH3(-CH4)), and a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit ($VH_{(1)}$ -CH1₍₁₎-CH2-CH3(-CH4)). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule ($VH_{(2)}$ -CL₍₂₎) and the Fab light chain polypeptide of the first Fab molecule ($VL_{(1)}$ -CL₍₁₎). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit ($VH_{(2)}$ -CL₍₂₎-CH2-CH3(-CH4)), and a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit ($VH_{(1)}$ -CH1₍₁₎-CH2-CH3(-CH4)). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule ($VL_{(2)}$ -CH1₍₂₎) and the Fab light chain polypeptide of the first Fab molecule ($VL_{(1)}$ -CL₍₁₎). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

In some embodiments, the T cell activating bispecific antigen binding molecule comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a

carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn
 5 shares a carboxy-terminal peptide bond with an Fc domain subunit (VL₍₂₎-CH1₍₂₎-VH₍₁₎-CH1₍₁₎-CH2-CH3(-CH4)). In other embodiments, the T cell activating bispecific antigen binding molecule comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain
 10 constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VH₍₁₎-CH1₍₁₎-VL₍₂₎-CH1₍₂₎-CH2-CH3(-CH4)).

In some of these embodiments the T cell activating bispecific antigen binding molecule further
 15 comprises a crossover Fab light chain polypeptide of the second Fab molecule, wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH₍₂₎-CL₍₂₎), and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In others of these embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein
 20 the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain polypeptide of the first Fab molecule (VH₍₂₎-CL₍₂₎-VL₍₁₎-CL₍₁₎), or a polypeptide wherein the Fab light chain polypeptide of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable
 25 region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VL₍₁₎-CL₍₁₎-VH₍₂₎-CL₍₂₎), as appropriate.

The T cell activating bispecific antigen binding molecule according to these embodiments may further comprise (i) an Fc domain subunit polypeptide (CH2-CH3(-CH4)), or (ii) a polypeptide
 30 wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit (VH₍₃₎-CH1₍₃₎-CH2-CH3(-CH4)) and the Fab light chain polypeptide of a third Fab molecule (VL₍₃₎-CL₍₃₎). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

In some embodiments, the T cell activating bispecific antigen binding molecule comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VH₍₂₎-CL₍₂₎-VH₍₁₎-CH1₍₁₎-CH2-CH3(-CH4)). In other embodiments, the T cell activating bispecific antigen binding molecule comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with an Fc domain subunit (VH₍₁₎-CH1₍₁₎-VH₍₂₎-CL₍₂₎-CH2-CH3(-CH4)).

In some of these embodiments the T cell activating bispecific antigen binding molecule further comprises a crossover Fab light chain polypeptide of the second Fab molecule, wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL₍₂₎-CH1₍₂₎), and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In others of these embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain polypeptide of the first Fab molecule (VL₍₂₎-CH1₍₂₎-VL₍₁₎-CL₍₁₎), or a polypeptide wherein the Fab light chain polypeptide of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VL₍₁₎-CL₍₁₎-VH₍₂₎-CL₍₂₎), as appropriate. The T cell activating bispecific antigen binding molecule according to these embodiments may further comprise (i) an Fc domain subunit polypeptide (CH2-CH3(-CH4)), or (ii) a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with an Fc domain subunit (VH₍₃₎-CH1₍₃₎-CH2-CH3(-CH4)) and the Fab light chain polypeptide

of a third Fab molecule ($VL_{(3)}-CL_{(3)}$). In certain embodiments the polypeptides are covalently linked, e.g., by a disulfide bond.

In some embodiments, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In certain such
5 embodiments, the T cell activating bispecific antigen binding molecule does not comprise an Fc domain. In certain embodiments, the T cell activating bispecific antigen binding molecule essentially consists of the first and the second Fab molecule, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. Such a configuration is
10 schematically depicted in Figures 1O and 1S.

In other embodiments, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. In certain such
embodiments, the T cell activating bispecific antigen binding molecule does not comprise an Fc domain. In certain embodiments, the T cell activating bispecific antigen binding molecule
15 essentially consists of the first and the second Fab molecule, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. Such a configuration is schematically depicted in Figures 1P and 1T.

In some embodiments, the first Fab molecule is fused at the C-terminus of the Fab heavy chain
20 to the N-terminus of the Fab heavy chain of the second Fab molecule, and the T cell activating bispecific antigen binding molecule further comprises a third Fab molecule, wherein said third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. In particular such embodiments, said third Fab molecule is a conventional Fab molecule. In other such embodiments, said third Fab molecule is a crossover
25 Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CL and $CH1$ of the Fab heavy and light chains are exchanged / replaced by each other. In certain such embodiments, the T cell activating bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab
30 heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule. Such a configuration is schematically depicted in Figure

1Q and 1U (particular embodiments, wherein the third Fab molecule is a conventional Fab molecule and preferably identical to the first Fab molecule).

In some embodiments, the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule, and the T cell activating
5 bispecific antigen binding molecule further comprises a third Fab molecule, wherein said third Fab molecule is fused at the N-terminus of the Fab heavy chain to the C-terminus of the Fab heavy chain of the second Fab molecule. In particular such embodiments, said third Fab molecule is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light
10 chains are exchanged / replaced by each other. In other such embodiments, said third Fab molecule is a conventional Fab molecule. In certain such embodiments, the T cell activating bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, and optionally one or more peptide linkers, wherein the first Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second
15 Fab molecule, and the third Fab molecule is fused at the N-terminus of the Fab heavy chain to the C-terminus of the Fab heavy chain of the second Fab molecule. Such a configuration is schematically depicted in Figure 1W and 1Y (particular embodiments, wherein the third Fab molecule is a crossover Fab molecule and preferably identical to the second Fab molecule).

In some embodiments, the second Fab molecule is fused at the C-terminus of the Fab heavy
20 chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the T cell activating bispecific antigen binding molecule further comprises a third Fab molecule, wherein said third Fab molecule is fused at the N-terminus of the Fab heavy chain to the C-terminus of the Fab heavy chain of the first Fab molecule. In particular such embodiments, said third Fab molecule is a conventional Fab molecule. In other such embodiments, said third Fab molecule is
25 a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other. In certain such embodiments, the T cell activating bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-
30 terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the third Fab molecule is fused at the N-terminus of the Fab heavy chain to the C-terminus of the Fab heavy chain of the first Fab molecule. Such a configuration is schematically

depicted in Figure 1R and 1V (particular embodiments, wherein the third Fab molecule is a conventional Fab molecule and preferably identical to the first Fab molecule).

In some embodiments, the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the T cell activating bispecific antigen binding molecule further comprises a third Fab molecule, wherein said third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. In particular such embodiments, said third Fab molecule is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other. In other such embodiments, said third Fab molecule is a conventional Fab molecule. In certain such embodiments, the T cell activating bispecific antigen binding molecule essentially consists of the first, the second and the third Fab molecule, and optionally one or more peptide linkers, wherein the second Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the first Fab molecule, and the third Fab molecule is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab heavy chain of the second Fab molecule. Such a configuration is schematically depicted in Figure 1X and 1Z (particular embodiments, wherein the third Fab molecule is a crossover Fab molecule and preferably identical to the first Fab molecule).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region) (VH₍₁₎-CH1₍₁₎-VL₍₂₎-CH1₍₂₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH₍₂₎-CL₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain,

wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule (VL₍₂₎-CH1₍₂₎-VH₍₁₎-CH1₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH₍₂₎-CL₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule (VH₍₂₎-CL₍₂₎-VH₍₁₎-CH1₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL₍₂₎-CH1₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region) (VH₍₃₎-CH1₍₃₎-VH₍₁₎-CH1₍₁₎-VL₍₂₎-CH1₍₂₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH₍₂₎-CL₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL₍₃₎-CL₍₃₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region) (VH₍₃₎-CH1₍₃₎-VH₍₁₎-CH1₍₁₎-VH₍₂₎-CL₍₂₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL₍₂₎-CH1₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL₍₃₎-CL₍₃₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of a third Fab molecule (VL₍₂₎-CH1₍₂₎-VH₍₁₎-CH1₍₁₎-VH₍₃₎-CH1₍₃₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH₍₂₎-CL₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL₍₃₎-CL₍₃₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in

turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of a third Fab molecule (VH₍₂₎-CL₍₂₎-VH₍₁₎-CH1₍₁₎-VH₍₃₎-CH1₍₃₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL₍₂₎-CH1₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises the Fab light chain polypeptide of a third Fab molecule (VL₍₃₎-CL₍₃₎).

10 In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab light chain variable region of a third Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region) (VH₍₁₎-CH1₍₁₎-VL₍₂₎-CH1₍₂₎-VL₍₃₎-CH1₍₃₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (VH₍₂₎-CL₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule (VH₍₃₎-CL₍₃₎).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain of the first Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant

region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of a third Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region) ($VH_{(1)}-CH1_{(1)}-VH_{(2)}-CL_{(2)}-VH_{(3)}-CL_{(3)}$). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule ($VL_{(2)}-CH1_{(2)}$) and the Fab light chain polypeptide of the first Fab molecule ($VL_{(1)}-CL_{(1)}$). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule ($VL_{(3)}-CH1_{(3)}$).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab light chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab light chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain variable region is replaced by a light chain variable region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule ($VL_{(3)}-CH1_{(3)}-VL_{(2)}-CH1_{(2)}-VH_{(1)}-CH1_{(1)}$). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule ($VH_{(2)}-CL_{(2)}$) and the Fab light chain polypeptide of the first Fab molecule ($VL_{(1)}-CL_{(1)}$). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab heavy chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of a third Fab molecule ($VH_{(3)}-CL_{(3)}$).

In certain embodiments the T cell activating bispecific antigen binding molecule according to the invention comprises a polypeptide wherein the Fab heavy chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab light chain constant region of a

third Fab molecule (i.e. the third Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain variable region of the second Fab molecule, which in turn shares a carboxy-terminal peptide bond with the Fab light chain constant region of the second Fab molecule (i.e. the second Fab molecule comprises a crossover Fab heavy chain, wherein the heavy chain constant region is replaced by a light chain constant region), which in turn shares a carboxy-terminal peptide bond with the Fab heavy chain of the first Fab molecule (VH₍₃₎-CL₍₃₎-VH₍₂₎-CL₍₂₎-VH₍₁₎-CH1₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of the second Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of the second Fab molecule (VL₍₂₎-CH1₍₂₎) and the Fab light chain polypeptide of the first Fab molecule (VL₍₁₎-CL₍₁₎). In some embodiments the T cell activating bispecific antigen binding molecule further comprises a polypeptide wherein the Fab light chain variable region of a third Fab molecule shares a carboxy-terminal peptide bond with the Fab heavy chain constant region of a third Fab molecule (VL₍₃₎-CH1₍₃₎).

According to any of the above embodiments, components of the T cell activating bispecific antigen binding molecule (e.g. Fab molecules, Fc domain) may be fused directly or through various linkers, particularly peptide linkers comprising one or more amino acids, typically about 2-20 amino acids, that are described herein or are known in the art. Suitable, non-immunogenic peptide linkers include, for example, (G₄S)_n, (SG₄)_n, (G₄S)_n or G₄(SG₄)_n peptide linkers, wherein n is generally an integer from 1 to 10, typically from 2 to 4.

Fc domain

The Fc domain of the T cell activating bispecific antigen binding molecule consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other. In one embodiment the T cell activating bispecific antigen binding molecule of the invention comprises not more than one Fc domain.

In one embodiment according to the invention the Fc domain of the T cell activating bispecific antigen binding molecule is an IgG Fc domain. In a particular embodiment the Fc domain is an IgG₁ Fc domain. In another embodiment the Fc domain is an IgG₄ Fc domain. In a more specific

embodiment, the Fc domain is an IgG₄ Fc domain comprising an amino acid substitution at position S228 (Kabat numbering), particularly the amino acid substitution S228P. This amino acid substitution reduces in vivo Fab arm exchange of IgG₄ antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). In a further particular embodiment the Fc domain is human. An exemplary sequence of a human IgG₁ Fc region is given in SEQ ID NO: 13.

Fc domain modifications promoting heterodimerization

T cell activating bispecific antigen binding molecules according to the invention comprise different Fab molecules, fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain are typically comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of T cell activating bispecific antigen binding molecules in recombinant production, it will thus be advantageous to introduce in the Fc domain of the T cell activating bispecific antigen binding molecule a modification promoting the association of the desired polypeptides.

Accordingly, in particular embodiments the Fc domain of the T cell activating bispecific antigen binding molecule according to the invention comprises a modification promoting the association of the first and the second subunit of the Fc domain. The site of most extensive protein-protein interaction between the two subunits of a human IgG Fc domain is in the CH3 domain of the Fc domain. Thus, in one embodiment said modification is in the CH3 domain of the Fc domain.

There exist several approaches for modifications in the CH3 domain of the Fc domain in order to enforce heterodimerization, which are well described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304, WO 2011/90754, WO 2011/143545, WO 2012058768, WO 2013157954, WO 2013096291.

Typically, in all such approaches the CH3 domain of the first subunit of the Fc domain and the CH3 domain of the second subunit of the Fc domain are both engineered in a complementary manner so that each CH3 domain (or the heavy chain comprising it) can no longer homodimerize with itself but is forced to heterodimerize with the complementarily engineered other CH3 domain (so that the first and second CH3 domain heterodimerize and no homodimers between the two first or the two second CH3 domains are formed). These different approaches for improved heavy chain heterodimerization are contemplated as different alternatives in combination with the heavy-light chain modifications (VH and VL exchange/replacement in one binding arm and the introduction of substitutions of charged amino acids with opposite charges in the CH1/CL

interface) in the T cell activating bispecific antigen binding molecule according to the invention which reduce light chain mispairing and Bence Jones-type side products.

In a specific embodiment said modification promoting the association of the first and the second subunit of the Fc domain is a so-called “knob-into-hole” modification, comprising a “knob”
5 modification in one of the two subunits of the Fc domain and a “hole” modification in the other one of the two subunits of the Fc domain.

The knob-into-hole technology is described e.g. in US 5,731,168; US 7,695,936; Ridgway et al., Prot Eng 9, 617-621 (1996) and Carter, J Immunol Meth 248, 7-15 (2001). Generally, the method involves introducing a protuberance (“knob”) at the interface of a first polypeptide and a
10 corresponding cavity (“hole”) in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (e.g. tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the
15 interface of the second polypeptide by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine).

Accordingly, in a particular embodiment, in the CH3 domain of the first subunit of the Fc domain of the T cell activating bispecific antigen binding molecule an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a
20 protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue 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.

25 Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine (R), phenylalanine (F), tyrosine (Y), and tryptophan (W).

Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V).

The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides,
30 e.g. by site-specific mutagenesis, or by peptide synthesis.

In a specific embodiment, in the CH3 domain of the first subunit of the Fc domain (the “knobs” subunit) the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the Fc domain (the “hole” subunit) the tyrosine

residue at position 407 is replaced with a valine residue (Y407V). In one embodiment, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numberings according to Kabat EU index).

- 5 In yet a further embodiment, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index). Introduction of these two cysteine residues results in
10 formation of a disulfide bridge between the two subunits of the Fc domain, further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

In a particular embodiment, the first subunit of the Fc domain comprises amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).

- 15 In a particular embodiment the Fab molecule which specifically binds an activating T cell antigen is fused (optionally via a Fab molecule which specifically binds to a target cell antigen) to the first subunit of the Fc domain (comprising the “knob” modification). Without wishing to be bound by theory, fusion of the Fab molecule which specifically binds an activating T cell antigen to the knob-containing subunit of the Fc domain will (further) minimize the generation
20 of antigen binding molecules comprising two Fab molecules which bind to an activating T cell antigen (steric clash of two knob-containing polypeptides).

- Other techniques of CH3-modification for enforcing the heterodimerization are contemplated as alternatives according to the invention and are described e.g. in WO 96/27011, WO 98/050431, EP 1870459, WO 2007/110205, WO 2007/147901, WO 2009/089004, WO 2010/129304,
25 WO 2011/90754, WO 2011/143545, WO 2012/058768, WO 2013/157954, WO 2013/096291.

- In one embodiment the heterodimerization approach described in EP 1870459 A1, is used alternatively. This approach is based on the introduction of charged amino acids with opposite charges at specific amino acid positions in the CH3/CH3 domain interface between the two subunits of the Fc domain. One preferred embodiment for the T cell activating bispecific antigen
30 binding molecule of the invention are amino acid mutations R409D; K370E in one of the two CH3 domains (of the Fc domain) and amino acid mutations D399K; E357K in the other one of the CH3 domains of the Fc domain (numbering according to Kabat EU index).

In another embodiment the T cell activating bispecific antigen binding molecule of the invention comprises amino acid mutation T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (numberings according to Kabat EU index).

In another embodiment T cell activating bispecific antigen binding molecule of the invention comprises amino acid mutations S354C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations Y349C, T366S, L368A, Y407V in the CH3 domain of the second subunit of the Fc domain, or said T cell activating bispecific antigen binding molecule comprises amino acid mutations Y349C, T366W in the CH3 domain of the first subunit of the Fc domain and amino acid mutations S354C, T366S, L368A, Y407V in the CH3 domains of the second subunit of the Fc domain and additionally amino acid mutations R409D; K370E in the CH3 domain of the first subunit of the Fc domain and amino acid mutations D399K; E357K in the CH3 domain of the second subunit of the Fc domain (all numberings according to Kabat EU index).

In one embodiment the heterodimerization approach described in WO 2013/157953 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutation T366K and a second CH3 domain comprises amino acid mutation L351D (numberings according to Kabat EU index). In a further embodiment the first CH3 domain comprises further amino acid mutation L351K. In a further embodiment the second CH3 domain comprises further an amino acid mutation selected from Y349E, Y349D and L368E (preferably L368E) (numberings according to Kabat EU index).

In one embodiment the heterodimerization approach described in WO 2012/058768 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment the second CH3 domain comprises a further amino acid mutation at position T411, D399, S400, F405, N390, or K392, e.g. selected from a) T411N, T411R, T411Q, T411K, T411D, T411E or T411W, b) D399R, D399W, D399Y or D399K, c) S400E, S400D, S400R, or S400K, d) F405I, F405M, F405T, F405S, F405V or F405W, e) N390R, N390K or N390D, f) K392V, K392M, K392R, K392L, K392F or K392E (numberings according to Kabat EU index). In a further embodiment a first CH3 domain comprises amino acid mutations L351Y, Y407A and a second CH3 domain comprises amino acid mutations T366V, K409F. In a further

embodiment a first CH3 domain comprises amino acid mutation Y407A and a second CH3 domain comprises amino acid mutations T366A, K409F. In a further embodiment the second CH3 domain further comprises amino acid mutations K392E, T411E, D399R and S400R (numberings according to Kabat EU index).

- 5 In one embodiment the heterodimerization approach described in WO 2011/143545 is used alternatively, e.g. with the amino acid modification at a position selected from the group consisting of 368 and 409 (numbering according to Kabat EU index).

In one embodiment the heterodimerization approach described in WO 2011/090762, which also uses the knobs-into-holes technology described above, is used alternatively. In one embodiment
10 a first CH3 domain comprises amino acid mutation T366W and a second CH3 domain comprises amino acid mutation Y407A. In one embodiment a first CH3 domain comprises amino acid mutation T366Y and a second CH3 domain comprises amino acid mutation Y407T (numberings according to Kabat EU index).

In one embodiment the T cell activating bispecific antigen binding molecule or its Fc domain is
15 of IgG₂ subclass and the heterodimerization approach described in WO 2010/129304 is used alternatively.

In an alternative embodiment a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement
20 of one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable. In one such embodiment a first CH3 domain comprises amino acid substitution of K392 or N392 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K392D or N392D) and a second CH3 domain
25 comprises amino acid substitution of D399, E356, D356, or E357 with a positively charged amino acid (e.g. lysine (K) or arginine (R), preferably D399K, E356K, D356K, or E357K, and more preferably D399K and E356K). In a further embodiment the first CH3 domain further comprises amino acid substitution of K409 or R409 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D), preferably K409D or R409D). In a further embodiment
30 the first CH3 domain further or alternatively comprises amino acid substitution of K439 and/or K370 with a negatively charged amino acid (e.g. glutamic acid (E), or aspartic acid (D)) (all numberings according to Kabat EU index).

In yet a further embodiment the heterodimerization approach described in WO 2007/147901 is used alternatively. In one embodiment a first CH3 domain comprises amino acid mutations K253E, D282K, and K322D and a second CH3 domain comprises amino acid mutations D239K, E240K, and K292D (numberings according to Kabat EU index).

- 5 In still another embodiment the heterodimerization approach described in WO 2007/110205 can be used alternatively.

In one embodiment, the first subunit of the Fc domain comprises amino acid substitutions K392D and K409D, and the second subunit of the Fc domain comprises amino acid substitutions D356K and D399K (numbering according to Kabat EU index).

10 *Fc domain modifications reducing Fc receptor binding and/or effector function*

The Fc domain confers to the T cell activating bispecific antigen binding molecule favorable pharmacokinetic properties, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the T cell activating bispecific antigen binding molecule to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Moreover, the co-activation of Fc receptor signaling pathways may lead to cytokine release which, in combination with the T cell activating properties and the long half-life of the antigen binding molecule, results in excessive activation of cytokine receptors and severe side effects upon systemic administration. Activation of (Fc receptor-bearing) immune cells other than T cells may even reduce efficacy of the T cell activating bispecific antigen binding molecule due to the potential destruction of T cells e.g. by NK cells.

Accordingly, in particular embodiments, the Fc domain of the T cell activating bispecific antigen binding molecules according to the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain. In one such embodiment the Fc domain (or the T cell activating bispecific antigen binding molecule comprising said Fc domain) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG₁ Fc domain (or a T cell activating bispecific antigen binding molecule comprising a native IgG₁ Fc domain), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG₁ Fc domain (or a T cell activating bispecific antigen binding molecule comprising a native IgG₁ Fc domain). In one embodiment, the Fc domain (or the T cell activating bispecific antigen binding molecule comprising said Fc domain) does not substantially

bind to an Fc receptor and/or induce effector function. In a particular embodiment the Fc receptor is an Fc γ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fc γ receptor, more specifically human Fc γ RIIIa, Fc γ RI or Fc γ RIIa, most specifically human Fc γ RIIIa. In one embodiment the effector function is one or more selected from the group of CDC, ADCC, ADCP, and cytokine secretion. In a particular embodiment the effector function is ADCC. In one embodiment the Fc domain domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG₁ Fc domain domain. Substantially similar binding to FcRn is achieved when the Fc domain (or the T cell activating bispecific antigen binding molecule comprising said Fc domain) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG₁ Fc domain (or the T cell activating bispecific antigen binding molecule comprising a native IgG₁ Fc domain) to FcRn.

In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In particular embodiments, the Fc domain of the T cell activating bispecific antigen binding molecule comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the Fc domain to an Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the T cell activating bispecific antigen binding molecule comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to a T cell activating bispecific antigen binding molecule comprising a non-engineered Fc domain. In a particular embodiment the Fc receptor is an Fc γ receptor. In some embodiments the Fc receptor is a human Fc receptor. In some embodiments the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fc γ receptor, more specifically human Fc γ RIIIa, Fc γ RI or Fc γ RIIa, most specifically human Fc γ RIIIa. Preferably, binding to each of these receptors is

reduced. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the T cell activating bispecific antigen binding molecule comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or the T cell activating bispecific antigen binding molecule comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or T cell activating bispecific antigen binding molecules of the invention comprising said Fc domain, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc domain of the T cell activating bispecific antigen binding molecule is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced crosslinking of target-bound antibodies, reduced dendritic cell maturation, or reduced T cell priming. In one embodiment the reduced effector function is one or more selected from the group of reduced CDC, reduced ADCC, reduced ADCP, and reduced cytokine secretion. In a particular embodiment the reduced effector function is reduced ADCC. In one embodiment the reduced ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or a T cell activating bispecific antigen binding molecule comprising a non-engineered Fc domain).

In one embodiment the amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function is an amino acid substitution. In one embodiment the Fc domain comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In a more specific embodiment the Fc domain comprises an amino acid substitution at a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index). In some embodiments the Fc domain comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In one such embodiment, the Fc domain is an IgG₁ Fc domain, particularly a human IgG₁ Fc domain. In one embodiment the Fc domain comprises

an amino acid substitution at position P329. In a more specific embodiment the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In one embodiment the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331
5 (numberings according to Kabat EU index). In a more specific embodiment the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments the Fc domain comprises amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular embodiments the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA"). In one such
10 embodiment, the Fc domain is an IgG₁ Fc domain, particularly a human IgG₁ Fc domain. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor (as well as complement) binding of a human IgG₁ Fc domain, as described in PCT publication no. WO 2012/130831, incorporated herein by reference in its entirety. WO 2012/130831 also describes methods of preparing such mutant Fc domains and methods for
15 determining its properties such as Fc receptor binding or effector functions.

IgG₄ antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG₁ antibodies. Hence, in some embodiments the Fc domain of the T cell activating bispecific antigen binding molecules of the invention is an IgG₄ Fc domain, particularly a human IgG₄ Fc domain. In one embodiment the IgG₄ Fc domain comprises amino
20 acid substitutions at position S228, specifically the amino acid substitution S228P (numberings according to Kabat EU index). To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment the IgG₄ Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E (numberings according to Kabat EU index). In another embodiment, the IgG₄ Fc domain comprises an amino acid substitution at
25 position P329, specifically the amino acid substitution P329G (numberings according to Kabat EU index). In a particular embodiment, the IgG₄ Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G (numberings according to Kabat EU index). Such IgG₄ Fc domain mutants and their Fcγ receptor binding properties are described in PCT publication no. WO 2012/130831, incorporated
30 herein by reference in its entirety.

In a particular embodiment the Fc domain exhibiting reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain, is a human IgG₁ Fc domain comprising the amino acid substitutions L234A, L235A and optionally P329G, or a

human IgG₄ Fc domain comprising the amino acid substitutions S228P, L235E and optionally P329G (numberings according to Kabat EU index).

In certain embodiments N-glycosylation of the Fc domain has been eliminated. In one such embodiment the Fc domain comprises an amino acid mutation at position N297, particularly an amino acid substitution replacing asparagine by alanine (N297A) or aspartic acid (N297D) (numberings according to Kabat EU index).

In addition to the Fc domains described hereinabove and in PCT publication no. WO 2012/130831, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056) (numberings according to Kabat EU index). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

Mutant Fc domains can be prepared by amino acid deletion, substitution, insertion or modification using genetic or chemical methods well known in the art. Genetic methods may include site-specific mutagenesis of the encoding DNA sequence, PCR, gene synthesis, and the like. The correct nucleotide changes can be verified for example by sequencing.

Binding to Fc receptors can be easily determined e.g. by ELISA, or by Surface Plasmon Resonance (SPR) using standard instrumentation such as a BIAcore instrument (GE Healthcare), and Fc receptors such as may be obtained by recombinant expression. A suitable such binding assay is described herein. Alternatively, binding affinity of Fc domains or cell activating bispecific antigen binding molecules comprising an Fc domain for Fc receptors may be evaluated using cell lines known to express particular Fc receptors, such as human NK cells expressing FcγIIIa receptor.

Effector function of an Fc domain, or a T cell activating bispecific antigen binding molecule comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S. Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector

cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

- 5 In some embodiments, binding of the Fc domain to a complement component, specifically to C1q, is reduced. Accordingly, in some embodiments wherein the Fc domain is engineered to have reduced effector function, said reduced effector function includes reduced CDC. C1q binding assays may be carried out to determine whether the T cell activating bispecific antigen binding molecule is able to bind C1q and hence has CDC activity. See e.g., C1q and C3c binding
- 10 ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J Immunol Methods 202, 163 (1996); Cragg et al., Blood 101, 1045-1052 (2003); and Cragg and Glennie, Blood 103, 2738-2743 (2004)).

15 **Antigen Binding Moieties**

The antigen binding molecule of the invention is bispecific, i.e. it comprises at least two antigen binding moieties capable of specific binding to two distinct antigenic determinants. According to particular embodiments of the invention, the antigen binding moieties are Fab molecules (i.e. antigen binding domains composed of a heavy and a light chain, each comprising a variable and

20 a constant domain). In one embodiment said Fab molecules are human. In another embodiment said Fab molecules are humanized. In yet another embodiment said Fab molecules comprise human heavy and light chain constant domains.

Preferably, at least one of the antigen binding moieties is a crossover Fab molecule. Such modification reduces mispairing of heavy and light chains from different Fab molecules, thereby

25 improving the yield and purity of the T cell activating bispecific antigen binding molecule of the invention in recombinant production. In a particular crossover Fab molecule useful for the T cell activating bispecific antigen binding molecule of the invention, the variable domains of the Fab light chain and the Fab heavy chain (VL and VH, respectively) are exchanged. Even with this domain exchange, however, the preparation of the T cell activating bispecific antigen binding

30 molecule may comprise certain side products due to a so-called Bence Jones-type interaction between mispaired heavy and light chains (see Schaefer et al, PNAS, 108 (2011) 11187-11191). To further reduce mispairing of heavy and light chains from different Fab molecules and thus increase the purity and yield of the desired T cell activating bispecific antigen binding molecule,

according to the present invention charged amino acids with opposite charges may be introduced at specific amino acid positions in the CH1 and CL domains of either the Fab molecule(s) specifically binding to a target cell antigen, or the Fab molecule specifically binding to an activating T cell antigen. Charge modifications are made either in the conventional Fab molecule(s) comprised in the T cell activating bispecific antigen binding molecule (such as shown e.g. in Figures 1 A-C, G-J), or in the VH/VL crossover Fab molecule(s) comprised in the T cell activating bispecific antigen binding molecule (such as shown e.g. in Figure 1 D-F, K-N) (but not in both). In particular embodiments, the charge modifications are made in the conventional Fab molecule(s) comprised in the T cell activating bispecific antigen binding molecule (which in particular embodiments specifically bind(s) to the target cell antigen).

In a particular embodiment according to the invention, the T cell activating bispecific antigen binding molecule is capable of simultaneous binding to a target cell antigen, particularly a tumor cell antigen, and an activating T cell antigen, particularly CD3. In one embodiment, the T cell activating bispecific antigen binding molecule is capable of crosslinking a T cell and a target cell by simultaneous binding to a target cell antigen and an activating T cell antigen. In an even more particular embodiment, such simultaneous binding results in lysis of the target cell, particularly a tumor cell. In one embodiment, such simultaneous binding results in activation of the T cell. In other embodiments, such simultaneous binding results in a cellular response of a T lymphocyte, particularly a cytotoxic T lymphocyte, selected from the group of: proliferation, differentiation, cytokine secretion, cytotoxic effector molecule release, cytotoxic activity, and expression of activation markers. In one embodiment, binding of the T cell activating bispecific antigen binding molecule to the activating T cell antigen, particularly CD3, without simultaneous binding to the target cell antigen does not result in T cell activation.

In one embodiment, the T cell activating bispecific antigen binding molecule is capable of re-directing cytotoxic activity of a T cell to a target cell. In a particular embodiment, said re-direction is independent of MHC-mediated peptide antigen presentation by the target cell and and/or specificity of the T cell.

Particularly, a T cell according to any of the embodiments of the invention is a cytotoxic T cell. In some embodiments the T cell is a CD4⁺ or a CD8⁺ T cell, particularly a CD8⁺ T cell.

Activating T cell antigen binding moiety

The T cell activating bispecific antigen binding molecule of the invention comprises at least one antigen binding moiety, particularly a Fab molecule, which specifically binds to an activating T

cell antigen (also referred to herein as an “activating T cell antigen binding moiety, or activating T cell antigen binding Fab molecule”). In a particular embodiment, the T cell activating bispecific antigen binding molecule comprises not more than one antigen binding moiety capable of specific binding to an activating T cell antigen. In one embodiment the T cell activating
5 bispecific antigen binding molecule provides monovalent binding to the activating T cell antigen. In particular embodiments, the antigen binding moiety which specifically binds an activating T cell antigen is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other. In such embodiments, the antigen binding
10 moiety(ies) which specifically binds a target cell antigen is preferably a conventional Fab molecule. In embodiments where there is more than one antigen binding moiety, particularly Fab molecule, which specifically binds to a target cell antigen comprised in the T cell activating bispecific antigen binding molecule, the antigen binding moiety which specifically binds to an activating T cell antigen preferably is a crossover Fab molecule and the antigen binding moieties
15 which specifically bind to a target cell antigen are conventional Fab molecules.

In alternative embodiments, the antigen binding moiety which specifically binds an activating T cell antigen is a conventional Fab molecule. In such embodiments, the antigen binding moiety(ies) which specifically binds a target cell antigen is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant
20 domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other.

In a particular embodiment the activating T cell antigen is CD3, particularly human CD3 (SEQ ID NO: 1) or cynomolgus CD3 (SEQ ID NO: 2), most particularly human CD3. In a particular embodiment the activating T cell antigen binding moiety is cross-reactive for (i.e. specifically binds to) human and cynomolgus CD3. In some embodiments, the activating T cell antigen is the
25 epsilon subunit of CD3 (CD3 epsilon).

In some embodiments, the activating T cell antigen binding moiety specifically binds to CD3, particularly CD3 epsilon, and comprises at least one HVR-H selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 and at least one HVR-L selected from the group of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10.

30 In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the HVR-H 1 of SEQ ID NO: 4, the HVR-H 2 of SEQ ID NO: 5, the HVR-H 3 of SEQ ID NO: 6, and a light chain variable region

comprising the HVR-L 1 of SEQ ID NO: 8, the HVR-L 2 of SEQ ID NO: 9, and the HVR-L 3 of SEQ ID NO: 10.

In another embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the HVR-H 1 of SEQ ID NO: 4, the HVR-H 2 of SEQ ID NO: 28, the HVR-H 3 of SEQ ID NO: 6, and a light chain variable region comprising the HVR-L 1 of SEQ ID NO: 29, the HVR-L 2 of SEQ ID NO: 9, and the HVR-L 3 of SEQ ID NO: 10.

In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 3 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 7.

In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7.

In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises the heavy chain variable region sequence of SEQ ID NO: 3 and the light chain variable region sequence of SEQ ID NO: 7.

In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 30 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 31.

In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 30 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 31.

In one embodiment the CD3 binding antigen binding moiety, particularly Fab molecule, comprises the heavy chain variable region sequence of SEQ ID NO: 30 and the light chain variable region sequence of SEQ ID NO: 31.

Target cell antigen binding moiety

The T cell activating bispecific antigen binding molecule of the invention comprises at least one antigen binding moiety, particularly a Fab molecule, which specifically binds to Tim-3 (target cell antigen). In certain embodiments, the T cell activating bispecific antigen binding molecule comprises two antigen binding moieties, particularly Fab molecules, which specifically bind to

Tim-3. In a particular such embodiment, each of these antigen binding moieties specifically binds to the same antigenic determinant. In an even more particular embodiment, all of these antigen binding moieties are identical, i.e. they comprise the same amino acid sequences including the same amino acid substitutions in the CH1 and CL domain as described herein (if
5 any). In one embodiment, the T cell activating bispecific antigen binding molecule comprises an immunoglobulin molecule which specifically binds to Tim-3. In one embodiment the T cell activating bispecific antigen binding molecule comprises not more than two antigen binding moieties, particularly Fab molecules, which specifically bind to Tim-3.

In particular embodiments, the antigen binding moiety(ies) which specifically bind to Tim-3
10 is/are a conventional Fab molecule. In such embodiments, the antigen binding moiety(ies) which specifically binds an activating T cell antigen is a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other.

In alternative embodiments, the antigen binding moiety(ies) which specifically bind to Tim-3
15 is/are a crossover Fab molecule as described herein, i.e. a Fab molecule wherein the variable domains VH and VL or the constant domains CH1 and CL of the Fab heavy and light chains are exchanged / replaced by each other. In such embodiments, the antigen binding moiety(ies) which specifically binds an activating T cell antigen is a conventional Fab molecule.

The Tim-3 binding moiety is able to direct the T cell activating bispecific antigen binding
20 molecule to a target site, for example to a specific type of tumor cell that expresses Tim-3.

In one embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18
25 and the HVR-L 3 of SEQ ID NO: 19. In a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 20, and a light chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 21. In still a further embodiment, the antigen binding moiety,
30 particularly Fab molecule, which specifically binds to Tim-3 comprises the heavy chain variable region sequence of SEQ ID NO: 20, and the light chain variable region sequence of SEQ ID NO: 21. In one embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID

NO: 22, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 24, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 25. In a further embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide sequence of SEQ ID NO: 22, a polypeptide sequence of SEQ ID NO: 23, a polypeptide sequence of SEQ ID NO: 24 and a polypeptide sequence of SEQ ID NO: 25.

In one embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15, and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19. In a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 33, and a light chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 34. In still a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the heavy chain variable region sequence of SEQ ID NO: 33, and the light chain variable region sequence of SEQ ID NO: 34. In a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 35, and a light chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 36. In still a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the heavy chain variable region sequence of SEQ ID NO: 35, and the light chain variable region sequence of SEQ ID NO: 36.

In a particular embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 33, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 33, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 24, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 25 wherein the Tim-3 VL region

sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 34. In a further particular embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 33, a polypeptide sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 33, a polypeptide sequence of SEQ ID NO: 24 and a polypeptide sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 34. In another particular embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 35, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 35, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 24, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 36. In a further particular embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 35, a polypeptide sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 35, a polypeptide sequence of SEQ ID NO: 24 and a polypeptide sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 36.

In one embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38, and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42. In a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 43, and a light chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 44. In still a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the heavy chain variable

region sequence of SEQ ID NO: 43, and the light chain variable region sequence of SEQ ID NO: 44. In a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises a heavy chain variable region that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 45, and a light chain variable region
5 that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 46. In still a further embodiment, the antigen binding moiety, particularly Fab molecule, which specifically binds to Tim-3 comprises the heavy chain variable region sequence of SEQ ID NO: 45, and the light chain variable region sequence of SEQ ID NO: 46.

In a particular embodiment, the T cell activating bispecific antigen binding molecule comprises a
10 polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 43, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 43, a polypeptide that is at least 95%, 96%, 97%, 98%,
15 or 99% identical to the sequence of SEQ ID NO: 24, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 44. In a further particular embodiment, the T cell activating bispecific antigen binding molecule comprises a polypeptide sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 43, a polypeptide sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 43, a polypeptide sequence of SEQ ID NO: 24 and a polypeptide sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 44. In another particular embodiment, the T cell activating bispecific antigen binding
25 molecule comprises a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 45, a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 45, a polypeptide that is at least
30 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 24, and a polypeptide that is at least 95%, 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 46. In a further particular embodiment, the T cell activating bispecific antigen binding

molecule comprises a polypeptide sequence of SEQ ID NO: 22 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 45, a polypeptide sequence of SEQ ID NO: 23 wherein the Tim-3 VH region sequence (SEQ ID NO: 20) is replaced by the sequence of SEQ ID NO: 45, a polypeptide sequence of SEQ ID NO: 24 and a polypeptide sequence of SEQ ID NO: 25 wherein the Tim-3 VL region sequence (SEQ ID NO: 21) is replaced by the sequence of SEQ ID NO: 46.

Polynucleotides

The invention further provides isolated polynucleotides encoding a T cell activating bispecific antigen binding molecule as described herein or a fragment thereof. In some embodiments, said fragment is an antigen binding fragment.

The polynucleotides encoding T cell activating bispecific antigen binding molecules of the invention may be expressed as a single polynucleotide that encodes the entire T cell activating bispecific antigen binding molecule or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional T cell activating bispecific antigen binding molecule. For example, the light chain portion of a Fab molecule may be encoded by a separate polynucleotide from the portion of the T cell activating bispecific antigen binding molecule comprising the heavy chain portion of the Fab molecule, an Fc domain subunit and optionally (part of) another Fab molecule. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the Fab molecule. In another example, the portion of the T cell activating bispecific antigen binding molecule comprising one of the two Fc domain subunits and optionally (part of) one or more Fab molecules could be encoded by a separate polynucleotide from the portion of the T cell activating bispecific antigen binding molecule comprising the the other of the two Fc domain subunits and optionally (part of) a Fab molecule. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In some embodiments, the isolated polynucleotide encodes the entire T cell activating bispecific antigen binding molecule according to the invention as described herein. In other embodiments, the isolated polynucleotide encodes a polypeptides comprised in the T cell activating bispecific antigen binding molecule according to the invention as described herein.

In certain embodiments the polynucleotide or nucleic acid is DNA. In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA). RNA of the present invention may be single stranded or double stranded.

Recombinant Methods

T cell activating bispecific antigen binding molecules of the invention may be obtained, for example, by solid-state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the T cell activating bispecific antigen binding molecule (fragment), e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of a T cell activating bispecific antigen binding molecule (fragment) along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the T cell activating bispecific antigen binding molecule (fragment) (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the T cell activating bispecific antigen binding

molecule (fragment) of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit α -globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the T cell activating bispecific antigen binding molecule is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding a T cell activating bispecific antigen binding molecule of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.* an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β -glucuronidase.

DNA encoding a short protein sequence that could be used to facilitate later purification (*e.g.* a histidine tag) or assist in labeling the T cell activating bispecific antigen binding molecule may be included within or at the ends of the T cell activating bispecific antigen binding molecule (fragment) encoding polynucleotide.

In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (*e.g.* has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) a T cell activating bispecific antigen binding molecule of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the T cell activating bispecific antigen binding molecules of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of T cell activating bispecific antigen binding molecules are well known in the art. Such cells may be transfected or transduced as appropriate with the particular

expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the T cell activating bispecific antigen binding molecule for clinical applications. Suitable host cells include prokaryotic microorganisms, such as *E. coli*, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized”, resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, *Nat Biotech* 22, 1409-1414 (2004), and Li et al., *Nat Biotech* 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIESTM technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., *J Gen Virol* 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol Reprod* 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., *Annals N.Y. Acad Sci* 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr⁻ CHO cells (Urlaub et al., *Proc Natl Acad Sci USA* 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells

comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., Y0, NS0, Sp20 cell).

5 Standard technologies are known in the art to express foreign genes in these systems. Cells expressing a polypeptide comprising either the heavy or the light chain of an antigen binding domain such as an antibody, may be engineered so as to also express the other of the antibody chains such that the expressed product is an antibody that has both a heavy and a light chain.

In one embodiment, a method of producing a T cell activating bispecific antigen binding
10 molecule according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the T cell activating bispecific antigen binding molecule, as provided herein, under conditions suitable for expression of the T cell activating bispecific antigen binding molecule, and recovering the T cell activating bispecific antigen binding molecule from the host cell (or host cell culture medium).

15 The components of the T cell activating bispecific antigen binding molecule are genetically fused to each other. T cell activating bispecific antigen binding molecule can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between different
20 components of T cell activating bispecific antigen binding molecules are found in the sequences provided herein. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

In certain embodiments the one or more antigen binding moieties of the T cell activating
25 bispecific antigen binding molecules comprise at least an antibody variable region capable of binding an antigenic determinant. Variable regions can form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory manual", Cold Spring Harbor Laboratory, 1988). Non-naturally
30 occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty).

Any animal species of antibody, antibody fragment, antigen binding domain or variable region can be used in the T cell activating bispecific antigen binding molecules of the invention. Non-limiting antibodies, antibody fragments, antigen binding domains or variable regions useful in the present invention can be of murine, primate, or human origin. If the T cell activating

5 bispecific antigen binding molecule is intended for human use, a chimeric form of antibody may be used wherein the constant regions of the antibody are from a human. A humanized or fully human form of the antibody can also be prepared in accordance with methods well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs

10 onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but

15 "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front Biosci* 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332, 323-329 (1988); Queen et al., *Proc Natl Acad Sci USA* 86, 10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Jones et al., *Nature* 321, 522-525 (1986);

20 Morrison et al., *Proc Natl Acad Sci* 81, 6851-6855 (1984); Morrison and Oi, *Adv Immunol* 44, 65-92 (1988); Verhoeyen et al., *Science* 239, 1534-1536 (1988); Padlan, *Molec Immun* 31(3), 169-217 (1994); Kashmiri et al., *Methods* 36, 25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol Immunol* 28, 489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36, 43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36, 61-68 (2005) and

25 Klimka et al., *Br J Cancer* 83, 252-260 (2000) (describing the "guided selection" approach to FR shuffling). Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr Opin Pharmacol* 5, 368-74 (2001) and Lonberg, *Curr Opin Immunol* 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal

30 antibodies made by the hybridoma method (see e.g. *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact

antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, *Nat Biotech* 23, 1117-1125 (2005)). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in *Methods in Molecular Biology* 178, 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001); and McCafferty et al., *Nature* 348, 552-554; Clackson et al., *Nature* 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments.

In certain embodiments, the antigen binding moieties useful in the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the T cell activating bispecific antigen binding molecule of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (analyzed on a BIACORE T100 system) (Liljeblad, et al., *Glyco J* 17, 323-329 (2000)), and traditional binding assays (Heeley, *Endocr Res* 28, 217-229 (2002)). Competition assays may be used to identify an antibody, antibody fragment, antigen binding domain or variable domain that competes with a reference antibody for binding to a particular antigen, e.g. an antibody that competes with the V9 antibody for binding to CD3. In certain embodiments, such a competing antibody binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). In an exemplary competition assay, immobilized antigen (e.g. CD3) is incubated in a solution comprising a first labeled antibody that binds to the antigen (e.g. V9 antibody, described in US 6,054,297) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the antigen. The second antibody may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody

for binding to the antigen. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

T cell activating bispecific antigen binding molecules prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion
5 exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the T cell activating bispecific antigen binding
10 molecule binds. For example, for affinity chromatography purification of T cell activating bispecific antigen binding molecules of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate a T cell activating bispecific antigen binding molecule essentially as described in the Examples. The purity of the T cell activating bispecific antigen binding
15 molecule can be determined by any of a variety of well known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like. For example, the heavy chain fusion proteins expressed as described in the Examples were shown to be intact and properly assembled as demonstrated by reducing SDS-PAGE (see e.g. Figure 3). Three bands were resolved at approximately Mr 25,000, Mr 50,000 and Mr 75,000, corresponding to the predicted
20 molecular weights of the T cell activating bispecific antigen binding molecule light chain, heavy chain and heavy chain/light chain fusion protein.

Assays

T cell activating bispecific antigen binding molecules provided herein may be identified,
25 screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

Affinity assays

The affinity of the T cell activating bispecific antigen binding molecule for an Fc receptor or a
30 target antigen can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. Alternatively, binding of T cell activating bispecific antigen binding molecules for

different receptors or target antigens may be evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS). A specific illustrative and exemplary embodiment for measuring binding affinity is described in the following and in the Examples below.

- 5 According to one embodiment, K_D is measured by surface plasmon resonance using a BIACORE® T100 machine (GE Healthcare) at 25 °C.

To analyze the interaction between the Fc-portion and Fc receptors, His-tagged recombinant Fc-receptor is captured by an anti-Penta His antibody (Qiagen) immobilized on CM5 chips and the bispecific constructs are used as analytes. Briefly, carboxymethylated dextran biosensor chips
10 (CM5, GE Healthcare) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Anti Penta-His antibody is diluted with 10 mM sodium acetate, pH 5.0, to 40 µg/ml before injection at a flow rate of 5 µl/min to achieve approximately 6500 response units (RU) of coupled protein. Following the injection of the ligand, 1 M ethanolamine is injected to block
15 unreacted groups. Subsequently the Fc-receptor is captured for 60 s at 4 or 10 nM. For kinetic measurements, four-fold serial dilutions of the bispecific construct (range between 500 nM and 4000 nM) are injected in HBS-EP (GE Healthcare, 10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 0.05 % Surfactant P20, pH 7.4) at 25 °C at a flow rate of 30 µl/min for 120 s.

To determine the affinity to the target antigen, bispecific constructs are captured by an anti
20 human Fab specific antibody (GE Healthcare) that is immobilized on an activated CM5-sensor chip surface as described for the anti Penta-His antibody. The final amount of coupled protein is is approximately 12000 RU. The bispecific constructs are captured for 90 s at 300 nM. The target antigens are passed through the flow cells for 180 s at a concentration range from 250 to 1000 nM with a flowrate of 30 µl/min. The dissociation is monitored for 180 s.

- 25 Bulk refractive index differences are corrected for by subtracting the response obtained on reference flow cell. The steady state response was used to derive the dissociation constant K_D by non-linear curve fitting of the Langmuir binding isotherm. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® T100 Evaluation Software version 1.1.1) by simultaneously fitting the association
30 and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen et al., J Mol Biol 293, 865-881 (1999).

Activity assays

Biological activity of the T cell activating bispecific antigen binding molecules of the invention can be measured by various assays as described in the Examples. Biological activities may for example include the induction of proliferation of T cells, the induction of signaling in T cells, the induction of expression of activation markers in T cells, the induction of cytokine secretion by T cells, the induction of lysis of target cells such as tumor cells, and the induction of tumor regression and/or the improvement of survival.

Compositions, Formulations, and Routes of Administration

In a further aspect, the invention provides pharmaceutical compositions comprising any of the T cell activating bispecific antigen binding molecules provided herein, e.g., for use in any of the below therapeutic methods. In one embodiment, a pharmaceutical composition comprises any of the T cell activating bispecific antigen binding molecules provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical composition comprises any of the T cell activating bispecific antigen binding molecules provided herein and at least one additional therapeutic agent, e.g., as described below.

Further provided is a method of producing a T cell activating bispecific antigen binding molecule of the invention in a form suitable for administration in vivo, the method comprising (a) obtaining a T cell activating bispecific antigen binding molecule according to the invention, and (b) formulating the T cell activating bispecific antigen binding molecule with at least one pharmaceutically acceptable carrier, whereby a preparation of T cell activating bispecific antigen binding molecule is formulated for administration in vivo.

Pharmaceutical compositions of the present invention comprise a therapeutically effective amount of one or more T cell activating bispecific antigen binding molecule dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that are generally non-toxic to recipients at the dosages and concentrations employed, i.e. do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least one T cell activating bispecific antigen binding molecule and optionally an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity

standards as required by FDA Office of Biological Standards or corresponding authorities in other countries. Preferred compositions are lyophilized formulations or aqueous solutions. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, buffers, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.* antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, antioxidants, proteins, drugs, drug stabilizers, polymers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

The composition may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. T cell activating bispecific antigen binding molecules of the present invention (and any additional therapeutic agent) can be administered intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrasplenically, intrarenally, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctivally, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation (*e.g.* aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (*e.g.* liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference). Parenteral administration, in particular intravenous injection, is most commonly used for administering polypeptide molecules such as the T cell activating bispecific antigen binding molecules of the invention.

Parenteral compositions include those designed for administration by injection, *e.g.* subcutaneous, intradermal, intralesional, intravenous, intraarterial intramuscular, intrathecal or intraperitoneal injection. For injection, the T cell activating bispecific antigen binding molecules of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. The solution may contain formulatory agents such as suspending, stabilizing and/or dispersing

agents. Alternatively, the T cell activating bispecific antigen binding molecules may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. Sterile injectable solutions are prepared by incorporating the T cell activating bispecific antigen binding molecules of the invention in the required amount in the appropriate solvent with various
5 of the other ingredients enumerated below, as required. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the other ingredients. In the case of sterile powders for the preparation of sterile injectable solutions, suspensions or emulsion, the preferred methods of
10 preparation are vacuum-drying or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered liquid medium thereof. The liquid medium should be suitably buffered if necessary and the liquid diluent first rendered isotonic prior to injection with sufficient saline or glucose. The composition must be stable under the conditions of manufacture and storage, and preserved against the contaminating
15 action of microorganisms, such as bacteria and fungi. It will be appreciated that endotoxin contamination should be kept minimally at a safe level, for example, less than 0.5 ng/mg protein. Suitable pharmaceutically acceptable carriers include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride;
20 benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine;
25 monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Aqueous injection suspensions may contain compounds which increase the viscosity of the suspension, such as sodium
30 carboxymethyl cellulose, sorbitol, dextran, or the like. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic

solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl cleats or triglycerides, or liposomes.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-
5 microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences (18th Ed. Mack Printing Company, 1990). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include
10 semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, e.g. films, or microcapsules. In particular embodiments, prolonged absorption of an injectable composition can be brought about by the use in the compositions of agents delaying absorption, such as, for example, aluminum monostearate, gelatin or combinations thereof.

15 In addition to the compositions described previously, the T cell activating bispecific antigen binding molecules may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the T cell activating bispecific antigen binding molecules may be formulated with suitable polymeric or hydrophobic materials (for example as
20 an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

Pharmaceutical compositions comprising the T cell activating bispecific antigen binding molecules of the invention may be manufactured by means of conventional mixing, dissolving, emulsifying, encapsulating, entrapping or lyophilizing processes. Pharmaceutical compositions
25 may be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries which facilitate processing of the proteins into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

The T cell activating bispecific antigen binding molecules may be formulated into a composition
30 in a free acid or base, neutral or salt form. Pharmaceutically acceptable salts are salts that substantially retain the biological activity of the free acid or base. These include the acid addition salts, e.g., those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such

organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Pharmaceutical salts tend to be more soluble in aqueous and other protic solvents than are the corresponding free base forms.

Therapeutic Methods and Compositions

Any of the T cell activating bispecific antigen binding molecules provided herein may be used in therapeutic methods. T cell activating bispecific antigen binding molecules of the invention can be used as immunotherapeutic agents, for example in the treatment of cancers.

For use in therapeutic methods, T cell activating bispecific antigen binding molecules of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

In one aspect, T cell activating bispecific antigen binding molecules of the invention for use as a medicament are provided. In further aspects, T cell activating bispecific antigen binding molecules of the invention for use in treating a disease are provided. In certain embodiments, T cell activating bispecific antigen binding molecules of the invention for use in a method of treatment are provided. In one embodiment, the invention provides a T cell activating bispecific antigen binding molecule as described herein for use in the treatment of a disease in an individual in need thereof. In certain embodiments, the invention provides a T cell activating bispecific antigen binding molecule for use in a method of treating an individual having a disease comprising administering to the individual a therapeutically effective amount of the T cell activating bispecific antigen binding molecule. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. In further embodiments, the invention provides a T cell activating bispecific antigen binding molecule as described herein for use in inducing lysis of a target cell, particularly a tumor cell. In certain embodiments, the invention provides a T cell activating bispecific antigen binding molecule for use in a method of inducing lysis of a target cell,

particularly a cell, in an individual comprising administering to the individual an effective amount of the T cell activating bispecific antigen binding molecule to induce lysis of a target cell. An “individual” according to any of the above embodiments is a mammal, preferably a human.

- 5 In a further aspect, the invention provides for the use of a T cell activating bispecific antigen binding molecule of the invention in the manufacture or preparation of a medicament. In one embodiment the medicament is for the treatment of a disease in an individual in need thereof. In a further embodiment, the medicament is for use in a method of treating a disease comprising administering to an individual having the disease a therapeutically effective amount of the
- 10 medicament. In certain embodiments the disease to be treated is a proliferative disorder. In a particular embodiment the disease is cancer. In one embodiment, the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. In a further embodiment, the medicament is for inducing lysis of a target cell, particularly a tumor cell. In
- 15 still a further embodiment, the medicament is for use in a method of inducing lysis of a target cell, particularly a tumor cell, in an individual comprising administering to the individual an effective amount of the medicament to induce lysis of a target cell. An “individual” according to any of the above embodiments may be a mammal, preferably a human.

- In a further aspect, the invention provides a method for treating a disease. In one embodiment,
- 20 the method comprises administering to an individual having such disease a therapeutically effective amount of a T cell activating bispecific antigen binding molecule of the invention. In one embodiment a composition is administered to said individual, comprising the T cell activating bispecific antigen binding molecule of the invention in a pharmaceutically acceptable form. In certain embodiments the disease to be treated is a proliferative disorder. In a particular
- 25 embodiment the disease is cancer. In certain embodiments the method further comprises administering to the individual a therapeutically effective amount of at least one additional therapeutic agent, e.g., an anti-cancer agent if the disease to be treated is cancer. An “individual” according to any of the above embodiments may be a mammal, preferably a human.

- In a further aspect, the invention provides a method for inducing lysis of a target cell,
- 30 particularly a tumor cell. In one embodiment the method comprises contacting a target cell with a T cell activating bispecific antigen binding molecule of the invention in the presence of a T cell, particularly a cytotoxic T cell. In a further aspect, a method for inducing lysis of a target cell, particularly a tumor cell, in an individual is provided. In one such embodiment, the method

comprises administering to the individual an effective amount of a T cell activating bispecific antigen binding molecule to induce lysis of a target cell. In one embodiment, an "individual" is a human.

In certain embodiments the disease to be treated is a proliferative disorder, particularly cancer.

5 Non-limiting examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that can be treated using a T cell activating
10 bispecific antigen binding molecule of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and
15 cancer metastases. In certain embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer, lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer and blood cancer. In one embodiment the cancer is a leukemia or a lymphoma. In one embodiment the cancer is selected from the group consisting of Non-Hodgkin lymphoma (NHL), multiple myeloma (MM), large cell immunoblastic lymphoma (LCIBL), acute myeloid
20 leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML). A skilled artisan readily recognizes that in many cases the T cell activating bispecific antigen binding molecule may not provide a cure but may only provide partial benefit. In some embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of T cell activating bispecific antigen binding
25 molecule that provides a physiological change is considered an "effective amount" or a "therapeutically effective amount". The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

In some embodiments, an effective amount of a T cell activating bispecific antigen binding molecule of the invention is administered to a cell. In other embodiments, a therapeutically
30 effective amount of a T cell activating bispecific antigen binding molecule of the invention is administered to an individual for the treatment of disease.

For the prevention or treatment of disease, the appropriate dosage of a T cell activating bispecific antigen binding molecule of the invention (when used alone or in combination with one or more

other additional therapeutic agents) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the type of T cell activating bispecific antigen binding molecule, the severity and course of the disease, whether the T cell activating bispecific antigen binding molecule is administered for preventive or therapeutic purposes, previous or concurrent therapeutic interventions, the patient's clinical history and response to the T cell activating bispecific antigen binding molecule, and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

The T cell activating bispecific antigen binding molecule is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg – 10 mg/kg) of T cell activating bispecific antigen binding molecule can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the T cell activating bispecific antigen binding molecule would be in the range from about 0.005 mg/kg to about 10 mg/kg. In other non-limiting examples, a dose may also comprise from about 1 microgram/kg body weight, about 5 microgram/kg body weight, about 10 microgram/kg body weight, about 50 microgram/kg body weight, about 100 microgram/kg body weight, about 200 microgram/kg body weight, about 350 microgram/kg body weight, about 500 microgram/kg body weight, about 1 milligram/kg body weight, about 5 milligram/kg body weight, about 10 milligram/kg body weight, about 50 milligram/kg body weight, about 100 milligram/kg body weight, about 200 milligram/kg body weight, about 350 milligram/kg body weight, about 500 milligram/kg body weight, to about 1000 mg/kg body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg body weight to about 100 mg/kg body weight, about 5 microgram/kg body weight to about 500 milligram/kg body weight, etc., can be administered, based on the numbers described above. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 5.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered

intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the T cell activating bispecific antigen binding molecule). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

The T cell activating bispecific antigen binding molecules of the invention will generally be used in an amount effective to achieve the intended purpose. For use to treat or prevent a disease condition, the T cell activating bispecific antigen binding molecules of the invention, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

For systemic administration, a therapeutically effective dose can be estimated initially from *in vitro* assays, such as cell culture assays. A dose can then be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture.

Such information can be used to more accurately determine useful doses in humans.

Initial dosages can also be estimated from *in vivo* data, e.g., animal models, using techniques that are well known in the art. One having ordinary skill in the art could readily optimize administration to humans based on animal data.

Dosage amount and interval may be adjusted individually to provide plasma levels of the T cell activating bispecific antigen binding molecules which are sufficient to maintain therapeutic effect. Usual patient dosages for administration by injection range from about 0.1 to 50 mg/kg/day, typically from about 0.5 to 1 mg/kg/day. Therapeutically effective plasma levels may be achieved by administering multiple doses each day. Levels in plasma may be measured, for example, by HPLC.

In cases of local administration or selective uptake, the effective local concentration of the T cell activating bispecific antigen binding molecules may not be related to plasma concentration. One having skill in the art will be able to optimize therapeutically effective local dosages without undue experimentation.

A therapeutically effective dose of the T cell activating bispecific antigen binding molecules described herein will generally provide therapeutic benefit without causing substantial toxicity. Toxicity and therapeutic efficacy of a T cell activating bispecific antigen binding molecule can be determined by standard pharmaceutical procedures in cell culture or experimental animals. Cell culture assays and animal studies can be used to determine the LD_{50} (the dose lethal to 50%

of a population) and the ED₅₀ (the dose therapeutically effective in 50% of a population). The dose ratio between toxic and therapeutic effects is the therapeutic index, which can be expressed as the ratio LD₅₀/ED₅₀. T cell activating bispecific antigen binding molecules that exhibit large therapeutic indices are preferred. In one embodiment, the T cell activating bispecific antigen binding molecule according to the present invention exhibits a high therapeutic index. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosages suitable for use in humans. The dosage lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon a variety of factors, e.g., the dosage form employed, the route of administration utilized, the condition of the subject, and the like. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see, e.g., Fingl et al., 1975, in: The Pharmacological Basis of Therapeutics, Ch. 1, p. 1, incorporated herein by reference in its entirety).

The attending physician for patients treated with T cell activating bispecific antigen binding molecules of the invention would know how and when to terminate, interrupt, or adjust administration due to toxicity, organ dysfunction, and the like. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated, with the route of administration, and the like. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency will also vary according to the age, body weight, and response of the individual patient.

Other Agents and Treatments

The T cell activating bispecific antigen binding molecules of the invention may be administered in combination with one or more other agents in therapy. For instance, a T cell activating bispecific antigen binding molecule of the invention may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in an individual in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is an immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic agent, an activator of cell apoptosis, or an agent

that increases the sensitivity of cells to apoptotic inducers. In a particular embodiment, the additional therapeutic agent is an anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an
5 antiangiogenic agent.

Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of T cell activating bispecific antigen binding molecule used, the type of disorder or treatment, and other factors discussed above. The T cell activating bispecific antigen binding molecules are generally
10 used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate compositions), and separate
15 administration, in which case, administration of the T cell activating bispecific antigen binding molecule of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. T cell activating bispecific antigen binding molecules of the invention can also be used in combination with radiation therapy.

20 **Articles of Manufacture**

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc.
25 The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a T cell
30 activating bispecific antigen binding molecule of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a T cell activating bispecific antigen binding molecule of the

invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

10

Examples

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

15 General methods

Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook et al., Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturers' instructions. General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E.A. et al., (1991) Sequences of Proteins of Immunological Interest, 5th ed., NIH Publication No. 91-3242.

20

DNA Sequencing

25 DNA sequences were determined by double strand sequencing.

Gene Synthesis

Desired gene segments where required were either generated by PCR using appropriate templates or were synthesized by Geneart AG (Regensburg, Germany) from synthetic oligonucleotides and PCR products by automated gene synthesis. In cases where no exact gene sequence was available, oligonucleotide primers were designed based on sequences from closest homologues and the genes were isolated by RT-PCR from RNA originating from the appropriate

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tissue. The gene segments flanked by singular restriction endonuclease cleavage sites were cloned into standard cloning / sequencing vectors. The plasmid DNA was purified from transformed bacteria and concentration determined by UV spectroscopy. The DNA sequence of the subcloned gene fragments was confirmed by DNA sequencing. Gene segments were designed with suitable restriction sites to allow sub-cloning into the respective expression vectors. All constructs were designed with a 5'-end DNA sequence coding for a leader peptide which targets proteins for secretion in eukaryotic cells.

Example 1

Preparation of anti-Tim-3 / anti-CD3 T cell bispecific (TCB) molecules

The following molecule was prepared in this example; a schematic illustration thereof is shown in Figure 2:

A. "2+1 IgG CrossFab, inverted" with charge modifications (VH/VL exchange in CD3 binder, charge modification in Tim-3 binders) (Figure 2, SEQ ID NOs 22-25).

The variable region of heavy and light chain DNA sequences were subcloned in frame with either the constant heavy chain or the constant light chain pre-inserted into the respective recipient mammalian expression vector. Protein expression is driven by an MPSV promoter and a synthetic polyA signal sequence is present at the 3' end of the CDS. In addition each vector contains an EBV OriP sequence.

The molecules were produced by co-transfecting HEK293-EBNA cells growing in suspension with the mammalian expression vectors using polyethylenimine (PEI). The cells were transfected with the corresponding expression vectors in a 1:2:1:1 ratio ("vector heavy chain (VH-CH1-VL-CH1-CH2-CH3)" : "vector light chain (VL-CL)" : "vector heavy chain (VH-CH1-CH2-CH3)" : "vector light chain (VH-CL)").

For transfection HEK293 EBNA cells were cultivated in suspension serum free in Excell culture medium containing 6 mM L-glutamine and 250 mg/l G418. For the production in 600 ml tubespin flasks (max. working volume 400 mL) 600 million HEK293 EBNA cells were seeded 24 hours before transfection. For transfection cells were centrifuged for 5 min at 210 x g and supernatant was replaced by 20 ml pre-warmed CD CHO medium. Expression vectors were mixed in 20 ml CD CHO medium to a final amount of 400 µg DNA. After addition of 1080 µl PEI solution (2.7 µg/ml) the mixture was vortexed for 15 s and subsequently incubated for 10 min at room temperature. Afterwards cells were mixed with the DNA/PEI solution, transferred to

a 600 ml tubespin flask and incubated for 3 hours at 37°C in an incubator with a 5% CO₂ atmosphere. After incubation, 360 ml Excell + 6 mM L-glutamine + 5 g/L Pepsoy + 1.0 mM VPA medium was added and cells were cultivated for 24 hours. One day after transfection 7% Feed 1 was added. After 7 days cultivation supernatant was collected for purification by centrifugation for 20 - 30 min at 3600 x g (Sigma 8K centrifuge), the solution was sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01% w/v was added. The solution was kept at 4°C.

The concentration of the molecules in the culture medium was determined by Protein A-HPLC. The basis of separation was binding of Fc-containing molecules to Protein A at pH 8.0 and step elution from pH 2.5. There were two mobile phases. These were Tris (10 mM) -glycine (50 mM) - NaCl (100 mM) buffers, identical except that they were adjusted to different pHs (8 and 2.5). The column body was an Upchurch 2x20 mm pre-column with an internal volume of ~63 µl packed with POROS 20A. 100 µl of each sample was injected on equilibrated material with a flow rate of 0.5 ml/min. After 0.67 minutes the sample was eluted with a pH step to pH 2.5. Quantitation is done by determination of 280 nm absorbance and calculation using a standard curve with a concentration range of human IgG₁ from 16 to 166 mg/l.

The secreted protein was purified from cell culture supernatants by affinity chromatography using Protein A affinity chromatography, followed by a size exclusion chromatographic step.

For affinity chromatography supernatant was loaded on a HiTrap Protein A HP column (CV = 5 mL, GE Healthcare) equilibrated with 25 ml 20 mM sodium phosphate, 20 mM sodium citrate, pH 7.5. Unbound protein was removed by washing with at least 10 column volumes 20 mM sodium phosphate, 20 mM sodium citrate, 0.5 M sodium chloride pH 7.5 followed by an additional wash step using 6 column volumes 10 mM sodium phosphate, 20 mM sodium citrate, 0.5 M sodium chloride pH 5.45. Column was washed subsequently with 20 ml 10 mM MES, 100 mM sodium chloride, pH 5.0 and target protein was eluted in 6 column volumes 20 mM sodium citrate, 100 mM sodium chloride, 100 mM glycine, pH 3.0. Protein solution was neutralized by adding 1/10 of 0.5M sodium phosphate, pH 8.0. Target protein was concentrated and filtrated prior loading on a HiLoad Superdex 200 column (GE Healthcare) equilibrated with 20 mM histidine, 140 mM sodium chloride, 0.01% Tween20, pH 6.0.

The protein concentration of purified protein sample was determined by measuring the optical density (OD) at 280 nm, using the molar extinction coefficient calculated on the basis of the amino acid sequence.

Purity and molecular weight of the molecule after the final purification step were analyzed by CE-SDS analyses in the presence and absence of a reducing agent. The Caliper LabChip GXII system (Caliper Lifescience) was used according to the manufacturer's instruction. 2 µg sample was used for the analysis.

- 5 The aggregate content of the molecule was analyzed using a TSKgel G3000 SW XL analytical size-exclusion column (Tosoh) in 25 mM K₂HPO₄, 125 mM NaCl, 200 mM L-arginine monohydrochloride, 0.02% (w/v) NaN₃, pH 6.7 running buffer at 25°C.

The final quality of the molecule was very good, with nearly 100% monomer content and 100% purity on CE-SDS (Table 1 and 2, Figure 3).

- 10 TABLE 1. Summary of production and purification of anti-Tim 3 / anti-CD3 TCB molecule with charge modifications.

Molecule	Titer [mg/l]	Recovery [%]	Yield [mg/l]	Analytical SEC (HMW/Monomer/LMW) [%]
A	85.9	7.2	15.42	0.23/ 99.77 /0

TABLE 2. CE-SDS analyses (non-reduced) of anti-Tim-3 / anti-CD3 TCB molecule with charge modifications.

Molecule	Peak #	Size [kDa]	Purity [%]
A	1	212.0	100

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Example 2

Expression level of TIM-3 on different hematological tumor cell lines

- The expression level of TIM3 on the surface of different lymphoid and myeloid cell lines was analyzed by FACS. Briefly, human TIM3 expressing B cell lymphoma cells (exemplified as Pfeiffer cells), multiple myeloma cells (exemplified as RPMI-8226 cells) and acute megakaryoblastic leukemia cells (exemplified as CMK cells) were incubated with human BD Fc Block (BD Pharmingen) for 15 min at room temperature to block unspecific binding. Subsequently, 4 x 10⁵ cells (50 µl/well) were put into 96 well-v bottom microwell plates and 50
- 20

μl /well of PE labeled anti-TIM3 (clone 344823, R&D) (10 $\mu\text{g}/\text{ml}$ in BD Staining buffer (BD Pharmingen)) were added and incubated for 1 h at 4°C. After washing and centrifugation, mean fluorescence intensity (MFI) signals of single and viable cells were analyzed using a FACSCanto flow cytometer (BD Biosciences).

- 5 The specific binding was calculated as follow:

Specific Binding [MFI] = Geom. Mean MFI_{sample} - Geom. Mean MFI_{isotype control}

Ratio of specific Binding [RFI] = Geom. Mean MFI_{sample} / Geom. Mean MFI_{isotype control}

The results are shown in Figure 4 (Binding to different lymphoid und myeloid cell lines) and Table 3.

- 10 TABLE 3. Summary of TIM3 expression in different hematological tumor cell lines.

Cell line*	Description	FACS (MFI)	FACS (RFI)	FACS Expression
Pfeiffer	DLBCL (NHL)	3609	51	moderate
RPMI-8226	Plasmacytoma (MM)	1790	23	moderate
SR	Large cell immunoblastic lymphoma (LCIBL)	1051	19	moderate
HDLM-2	HL	292	5	very low
Raji	B cell lymphoma (BL)	173	2	very low
Karpas-299	T cell non-Hodgkin lymphoma (NHL)	247	3	very low
CCRF-CEM	T-ALL	59	1	very low
MEG-01	CML	158	3	very low
CMK	Leukemia, acute megakaryoblastic (AML)	384	5	low
KG-1	AML	363	5	low
EOL-1*	AML	207	2	very low
Kasumi-1	AML	164	4	very low
TF-1	Leukemia, erythroid (AML)	229	3	very low
MOLM-13	AML	67	1	negative

* 24h pre-treated with 10 ng/ml PMA for up-regulation of TIM3

For the evaluation of the potency of cytotoxic activity of the TIM3 TCB molecule lymphoid and myeloid cell lines with different expression levels of TIM3 were selected.

Example 3

- 15 **Cytotoxic activity of TIM3 TCB molecule on TIM-3 expressing NHL, MM and AML cells**

The redirected T-cell cytotoxic activity of the TIM3 TCB molecule of Example 1 in comparison to a non-binding (MCSP-targeted) TCB was evaluated by LDH release, using human T cells,

which had been in vitro expanded from PBMCs and frozen until use, as effector cells and various TIM3-positive tumor cell lines as targets. PBMCs were isolated by Ficoll density gradient centrifugation. Briefly, 50 μ l/well 1:10 serial dilutions of the TIM3 TCB molecule, non-binding control (MCSP TCB) or appropriate medium were added to 3×10^4 (100 μ l/well in 96 well round bottom plate, in triplicate) recombinant CHO K1 cells stably expressing human TIM-3 or 3×10^4 (100 μ l/well in 96 well round bottom plate, in triplicate) human TIM3 expressing B cell lymphoma cells (exemplified as Pfeiffer cells) or multiple myeloma cells (exemplified as RPMI-8226 cells). Increasing concentrations (ranges from 300 nM to 3 pM, in triplicate) of TIM3 TCB or non-binding control molecule were incubated for 24 h or 66 h with various TIM3 expressing tumor cells as well as effector cells at effector to target cell (E:T) ratios of 1:1 or 3:1 at 37°C. Cell lysis was assessed by Cytotoxicity Detection Kit (Roche, # 11 644 793 001) according to the manufacturer's instruction. Measurement took place at 490/650 nm.

The cytotoxic activity was calculated as follows:

$$\text{Cytotoxicity [\%]} = ((\text{experimental value} - E_{\text{negative control}}) / (E_{\text{positive control}} - E_{\text{negative control}})) * 100$$

The results are shown in Figure 5 (Cytotoxic activity of TIM3 TCB molecule on TIM-3 expressing recombinant and MM cell lines) and Table 4.

TABLE 4. Summary of cytotoxic activity and in vitro potency of the TIM3 TCB molecule.

Cell Line	Disease	Expression (FACS)			Cytotoxic activity TCB (LDH)	
		MFI	RFI	Expression level	IC50 pM	Max. Inhib. %
Pfeiffer	NHBL	3609	51	moderate	10	100
RPMI-8226	MM	1790	23	moderate	100	75
SR	LCIBL	1051	19	moderate	100	73
CMK	AML	384	5	low	300	81
KG-1	AML	363	5	low	1000	60
Karpas-299	NHTCL	247	3	very low	500	60
EOL-1*	AML	207	2	very low	300	100
Kasumi-1	AML	164	4	very low	3000	73
TF-1	Leukemia, erythroid	229	3	very low	N.A.	30

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* 24h pre-treated with 10 ng/ml PMA with extensive washing (5x) prior adding to LDH release based cytotoxicity assay

The results show that the TIM3 TCB molecule effectively killed tumor cells expressing different levels of TIM3. The TIM3 TCB molecule demonstrated highly potent cytotoxic activity (IC50 range 10-1000 pM) in all selected cell lines expressing moderate to very low levels of TIM3 on their surface.

Example 4

Preparation of anti-Tim-3 / anti-CD3 T cell bispecific (TCB) molecules comprising humanized Tim-3 binders

10 TIM3 TCB molecules are prepared according to the method described in Example 1, wherein the variable regions of the antigen binding moiety/ies binding to Tim-3 are replaced by humanized heavy and light chain variable regions.

Humanization of the VH and VL domains of murine anti-Tim3-0016 variant (0018) and anti-Tim3_0028

15 Murine anti-Tim-3 antibodies anti-Tim3-0016 variant (0018) and anti-Tim3_0028 described in PCT publication no. WO 2016/071448 (incorporated herein by reference in its entirety) were humanized.

Based upon the amino acid sequence of the VH and VL domains of a) anti-Tim3 antibody Tim3_0016 variant (0018) wherein the glycosylation site in the HVR-L 2 was removed by an N
20 to S mutation (SEQ ID NOs 47 and 48, respectively), humanized anti-Tim3 antibody variants Tim3-0433 and Tim3-0434 were generated and based upon the amino acid sequence of the VH and VL domains of b) anti-Tim3 antibody Tim3_0028 (SEQ ID NOs 49 and 50, respectively), humanized anti-Tim3 antibody variants Tim3-0438 and Tim3-0443 were generated.

The humanized amino acid sequences for heavy and light chain variable regions of were
25 backtranslated in to DNA and the resulting cNDA were synthesized (GenArt) and then cloned into heavy chain expression vectors as fusion proteins with human IgG1 backbones /human CH1-Hinge-CH2-CH3 with LALA and PG mutations (Leucine 234 to Alanine, Leucine 235 to Alanine, Proline 329 to Glycine) abrogating effector functions or into light chain expression vectors as fusion proteins to human C-kappa. LC and HC Plasmids were then cotransfected into

HEK293 and purified after 7 days from supernatants by standard methods for antibody purification. The resulting humanized Tim3-antibodies are named as follows:

TABLE 5. VH and VL sequences of humanized antibodies.

Humanized antibodies of Tim3_0016 variant (0018)	VH/SEQ ID NO:	VL/SEQ ID NO:
Tim3-0433	SEQ ID NO: 33	SEQ ID NO: 34
Tim3-0434	SEQ ID NO: 35	SEQ ID NO: 36
Humanized antibodies of Tim3_0028	VH/SEQ ID NO:	VL/SEQ ID NO:
Tim3-0438	SEQ ID NO: 43	SEQ ID NO: 44
Tim3-0443	SEQ ID NO: 45	SEQ ID NO: 46

TABLE 6. HVR sequences of humanized antibodies.

Humanized antibodies of Tim3_0016 variant (0018)	HVR-H1, HVR-H2, and HVR-H3 /SEQ ID NOs:	HVR-L1, HVR-L2, and HVR-L3 /SEQ ID NOs:
Tim3-0433	SEQ ID NOs: 14 , 15 and 16	SEQ ID NOs: 32 , 18 and 19
Tim3-0434	SEQ ID NOs: 14 , 15 and 16	SEQ ID NOs: 32 , 18 and 19
Humanized antibodies of Tim3_0028	HVR-H1, HVR-H2, and HVR-H3 /SEQ ID NOs:	HVR-L1, HVR-L2, and HVR-L3 /SEQ ID NOs:
Tim3-0438	SEQ ID NOs: 37 , 38 and 39	SEQ ID NOs: 40 , 41 and 42
Tim3-0443	SEQ ID NOs: 37 , 38 and 39	SEQ ID NOs: 40 , 41 and 42

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be

construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Claims

1. A T cell activating bispecific antigen binding molecule comprising

(a) a first antigen binding moiety which specifically binds to a first antigen;

(b) a second antigen binding moiety which specifically binds to a second antigen;

5 wherein the first antigen is an activating T cell antigen and the second antigen is Tim-3, or the first antigen is Tim-3 and the second antigen is an activating T cell antigen; and

wherein the antigen binding moiety which specifically binds to Tim-3 comprises (i) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 17, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, (ii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 14, the HVR-H 2 of SEQ ID NO: 15 and the HVR-H 3 of SEQ ID NO: 16, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 32, the HVR-L 2 of SEQ ID NO: 18 and the HVR-L 3 of SEQ ID NO: 19, or (iii) a heavy chain variable region, particularly a humanized heavy chain variable region, comprising the HVR-H 1 of SEQ ID NO: 37, the HVR-H 2 of SEQ ID NO: 38 and the HVR-H 3 of SEQ ID NO: 39, and a light chain variable region, particularly a humanized light chain variable region, comprising the HVR-L 1 of SEQ ID NO: 40, the HVR-L 2 of SEQ ID NO: 41 and the HVR-L 3 of SEQ ID NO: 42.

2. The T cell activating bispecific antigen binding molecule according to claim 1, wherein the antigen binding moiety which specifically binds to Tim-3 comprises (i) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 20 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 21, (ii) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 33 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 34, (iii) a heavy chain variable region

comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 35 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 36, (iv) a heavy chain variable region
5 comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 43 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 44, or (v) a heavy chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100%
10 identical to the amino acid sequence of SEQ ID NO: 45 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 46.

3. The T cell activating bispecific antigen binding molecule according to claim 1 or 2, wherein the first and/or the second antigen binding moiety is a Fab molecule.

15 4. The T cell activating bispecific antigen binding molecule according to any one of claims 1-3, wherein the second antigen binding moiety is a Fab molecule which specifically binds to a second antigen, and wherein the variable domains VL and VH or the constant domains CL and CH1 of the Fab light chain and the Fab heavy chain are replaced by each other.

5. The T cell activating bispecific antigen binding molecule according to any one of claims 1-4,
20 wherein the first antigen is Tim-3 and the second antigen is an activating T cell antigen.

6. The T cell activating bispecific antigen binding molecule according to any one of claims 1- 5, wherein the activating T cell antigen is CD3, particularly CD3 epsilon.

7. The T cell activating bispecific antigen binding molecule according to any one of claims 1-6, wherein the antigen binding moiety which specifically binds to the activating T cell antigen
25 comprises the HVR-H 1 of SEQ ID NO: 4, the HVR-H 2 of SEQ ID NO: 5, the HVR-H 3 of SEQ ID NO: 6, the HVR-L 1 of SEQ ID NO: 8, the HVR-L 2 of SEQ ID NO: 9 and the HVR-L 3 of SEQ ID NO: 10.

8. The T cell activating bispecific antigen binding molecule according to any one of claims 1-7, wherein the antigen binding moiety which specifically binds to the activating T cell antigen
30 comprises a heavy chain variable region comprising an amino acid sequence that is at least about

95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 3 and a light chain variable region comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 7.

9. The T cell activating bispecific antigen binding molecule according to any one of claims 1-8, wherein the first antigen binding moiety under (a) is a first Fab molecule which specifically binds to a first antigen, the second antigen binding moiety under (b) is a second Fab molecule which specifically binds to a second antigen wherein the variable domains VL and VH of the Fab light chain and the Fab heavy chain are replaced by each other;

and

- 10 i) in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule 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
- 15
- ii) in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule 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).
- 20

10. The T cell activating bispecific antigen binding molecule according to claim 9, wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule 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).

25

11. The T cell activating bispecific antigen binding molecule according to claim 9 or 10, wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to

30

Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

12. The T cell activating bispecific antigen binding molecule according to any one of claims 9-11, wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

13. The T cell activating bispecific antigen binding molecule according to any one of claims 9-12, wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

14. The T cell activating bispecific antigen binding molecule according to any one of claims 9-12, wherein in the constant domain CL of the first Fab molecule under a) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and wherein in the constant domain CH1 of the first Fab molecule under a) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

15. The T cell activating bispecific antigen binding molecule according to claim 9, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule 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).

16. The T cell activating bispecific antigen binding molecule according to claim 9 or 15, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

17. The T cell activating bispecific antigen binding molecule according to any one of claims 9, 15 and 16, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) and the amino acid at position 123 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted independently by glutamic acid (E), or aspartic acid (D) (numbering according to Kabat EU index).

18. The T cell activating bispecific antigen binding molecule according to any one of claims 9 and 15-17, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by arginine (R) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

19. The T cell activating bispecific antigen binding molecule according to any one of claims 9 and 15-17, wherein in the constant domain CL of the second Fab molecule under b) the amino acid at position 124 is substituted by lysine (K) (numbering according to Kabat) and the amino acid at position 123 is substituted by lysine (K) (numbering according to Kabat), and wherein in the constant domain CH1 of the second Fab molecule under b) the amino acid at position 147 is substituted by glutamic acid (E) (numbering according to Kabat EU index) and the amino acid at position 213 is substituted by glutamic acid (E) (numbering according to Kabat EU index).

20. The T cell activating bispecific antigen binding molecule according to any one of claims 1-19, further comprising

c) a third antigen binding moiety which specifically binds to the first antigen.

21. The T cell activating bispecific antigen binding molecule according to claim 20, wherein the
5 third antigen binding moiety is a Fab molecule.

22. The T cell activating bispecific antigen binding molecule according to claim 20 or 21, wherein the third antigen binding moiety is identical to the first antigen binding moiety.

23. The T cell activating bispecific antigen binding molecule according to any one of claims 20-22, wherein the first and the third antigen binding moiety specifically bind to a target cell antigen,
10 and the second antigen binding moiety specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon.

24. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 23, additionally comprising

d) an Fc domain composed of a first and a second subunit capable of stable association.

15 25. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 24, wherein the first and the second antigen binding moiety are fused to each other, optionally via a peptide linker.

26. The T cell activating bispecific antigen binding molecule according to any one of claims 1 to 25, wherein the first and the second antigen binding moieties are Fab molecules and the second
20 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.

27. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 25, wherein the first and the second antigen binding moieties are Fab molecules and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the Fab
25 heavy chain of the second antigen binding moiety.

28. The T cell activating bispecific antigen binding molecule of claim 26 or 27, wherein the first and the second antigen binding moieties are Fab molecules and the Fab light chain of the first

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

29. The T cell activating bispecific antigen binding molecule according to claim 24, wherein the first and the second antigen binding moieties are Fab molecules and the second antigen binding
5 moiety 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.

30. The T cell activating bispecific antigen binding molecule according to claim 24, wherein the first and the second antigen binding moieties are Fab molecules and the first antigen binding moiety is fused at the C-terminus of the Fab heavy chain to the N-terminus of the first or the
10 second subunit of the Fc domain.

31. The T cell activating bispecific antigen binding molecule according to claim 24, wherein the first and the second antigen binding moieties are Fab molecules and the first and the second 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.

15 32. The T cell activating bispecific antigen binding molecule according to any one of claims 24, 29 or 30, wherein the third antigen binding moiety is a Fab molecule and 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.

33. The T cell activating bispecific antigen binding molecule of claim 24, wherein the first, second and third antigen binding moieties are Fab molecules and the second and the third antigen
20 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.

34. The T cell activating bispecific antigen binding molecule according to claim 24, wherein the
25 first, second and third antigen binding moieties are Fab molecules and 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.

35. The T cell activating bispecific antigen binding molecule according to claim 34, wherein the first and the third antigen binding moiety and the Fc domain are part of an immunoglobulin molecule, particularly an IgG class immunoglobulin.

36. The T cell activating bispecific antigen binding molecule according to any one of claims 24-
5 35, wherein the Fc domain is an IgG, specifically an IgG₁ or IgG₄, Fc domain.

37. The T cell activating bispecific antigen binding molecule according to any one of claims 24-
36, wherein the Fc domain is a human Fc domain.

38. The T cell activating bispecific antigen binding molecule according to any one of claims 24-
37, wherein the Fc domain comprises a modification promoting the association of the first and
10 the second subunit of the Fc domain.

39. The T cell activating bispecific antigen binding molecule of claim 38, wherein in the CH3
domain of the first subunit of the Fc domain an amino acid residue 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
15 second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid
residue 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.

40. The T cell activating bispecific antigen binding molecule of claim 39, wherein said amino
20 acid residue having a larger side chain volume is selected from the group consisting of arginine
(R), phenylalanine (F), tyrosine (Y), and tryptophan (W), and said amino acid residue having a
smaller side chain volume is selected from the group consisting of alanine (A), serine (S),
threonine (T), and valine (V).

41. The T cell activating bispecific antigen binding molecule of claim 39 or 40, wherein in the
25 CH3 domain of the first subunit of the Fc domain the threonine residue at position 366 is
replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the
Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V), and
optionally in the second subunit of the Fc domain additionally the threonine residue at position
366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced
30 with an alanine residue (L368A) (numberings according to Kabat EU index).

42. The T cell activating bispecific antigen binding molecule of any one of claims 39-41, wherein in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numberings according to Kabat EU index).
43. The T cell activating bispecific antigen binding molecule of any one of claims 39-42, wherein the first subunit of the Fc domain comprises amino acid substitutions S354C and T366W, and the second subunit of the Fc domain comprises amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).
44. The T cell activating bispecific antigen binding molecule according to any one of claims 24-43, wherein the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG₁ Fc domain.
45. The T cell activating bispecific antigen binding molecule according to any one of claims 24-44, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.
46. The T cell activating bispecific antigen binding molecule according to claim 45, wherein said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering).
47. The T cell activating bispecific antigen binding molecule according to any one of claims 24-46, wherein each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an activating Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G (Kabat EU index numbering).
48. The T cell activating bispecific antigen binding molecule of any one of claims 44 to 47, wherein the Fc receptor is an Fcγ receptor.
49. The T cell activating bispecific antigen binding molecule of any one of claims 44 to 48, wherein the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).
50. One or more isolated polynucleotide encoding the T cell activating bispecific antigen binding molecule of any one of claims 1 to 49.

51. One or more vector, particularly expression vector, comprising the polynucleotide(s) of claim 50.

52. A host cell comprising the polynucleotide(s) of claim 50 or the vector(s) of claim 51.

53. A method of producing a T cell activating bispecific antigen binding molecule capable of
5 specific binding to Tim-3 and an activating T cell antigen, comprising the steps of a) culturing
the host cell of claim 52 under conditions suitable for the expression of the T cell activating
bispecific antigen binding molecule and b) optionally recovering the T cell activating bispecific
antigen binding molecule.

54. A T cell activating bispecific antigen binding molecule produced by the method of claim 53.

10 55. A pharmaceutical composition comprising the T cell activating bispecific antigen binding
molecule of any one of claims 1 to 49 or 54 and a pharmaceutically acceptable carrier.

56. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 49 or 54
or the pharmaceutical composition of claim 55 for use as a medicament.

57. The T cell activating bispecific antigen binding molecule of any one of claims 1 to 49 or 54
15 or the pharmaceutical composition of claim 55 for use in the treatment of a disease in an
individual in need thereof.

58. The T cell activating bispecific antigen binding molecule or the pharmaceutical composition
of claim 57, wherein the disease is cancer.

59. Use of the T cell activating bispecific antigen binding molecule of any one of claims 1 to 49
20 or 54 for the manufacture of a medicament for the treatment of a disease in an individual in need
thereof.

60. 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 of any one of claims 1 to 49 or 54 in a pharmaceutically acceptable
25 form.

61. The use of claim 59 or the method of claim 60, wherein said disease is cancer.

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62. A method for inducing lysis of a target cell, comprising contacting a target cell with the T cell activating bispecific antigen binding molecule of any one of claims 1-49 or 54 in the presence of a T cell.

63. The invention as described hereinbefore.

Figure 1

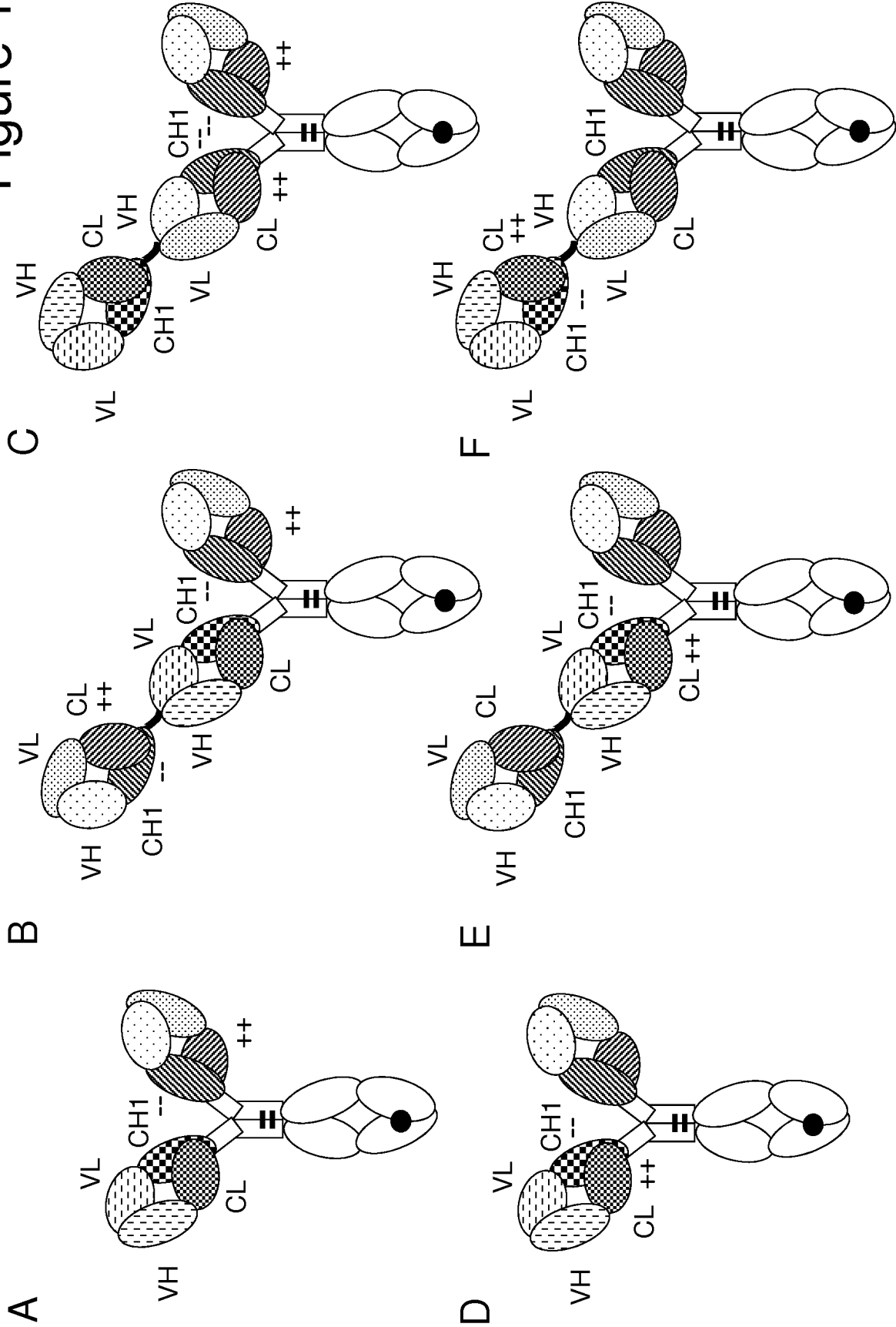


Figure 1

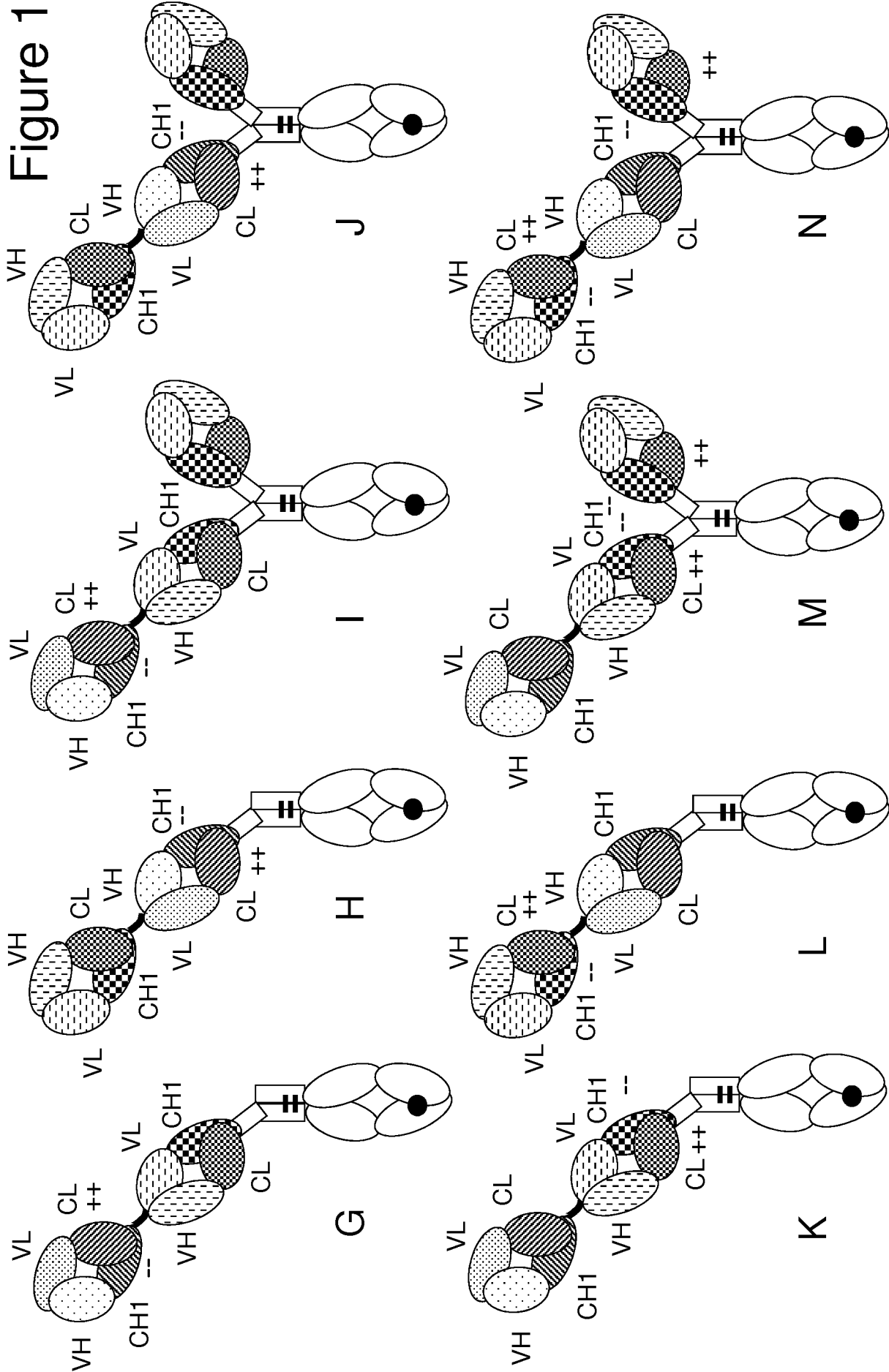


Figure 1

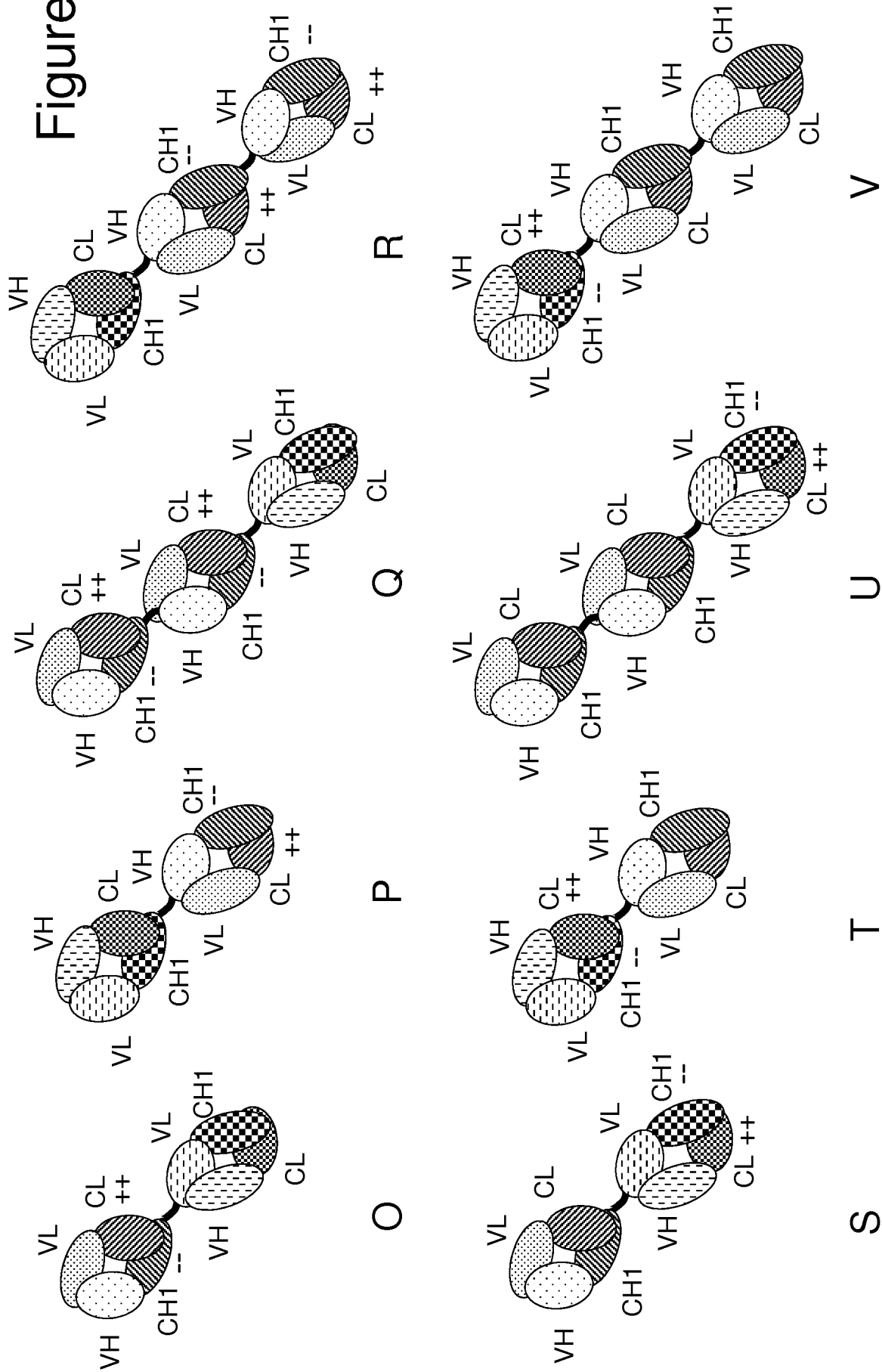
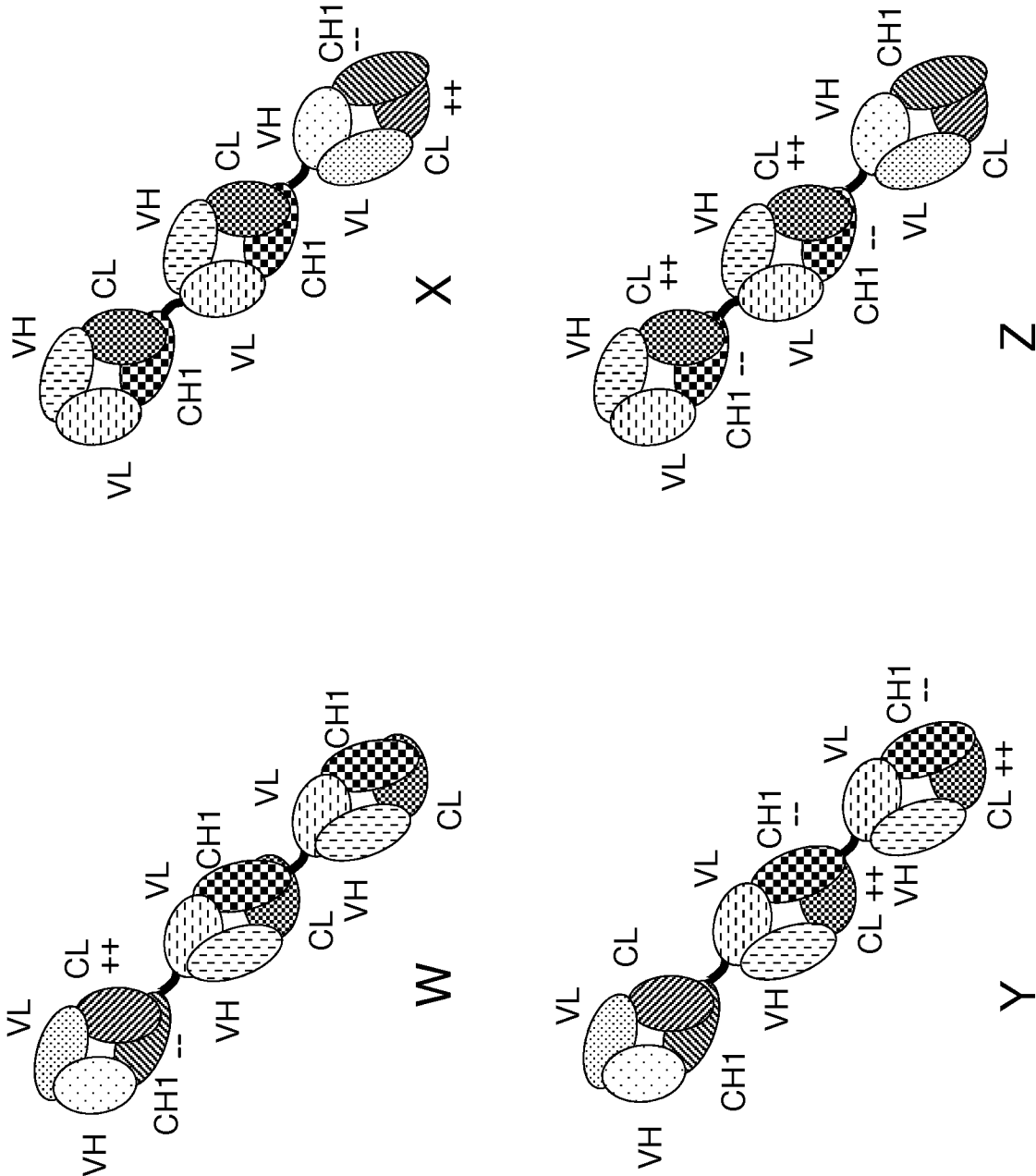


Figure 1



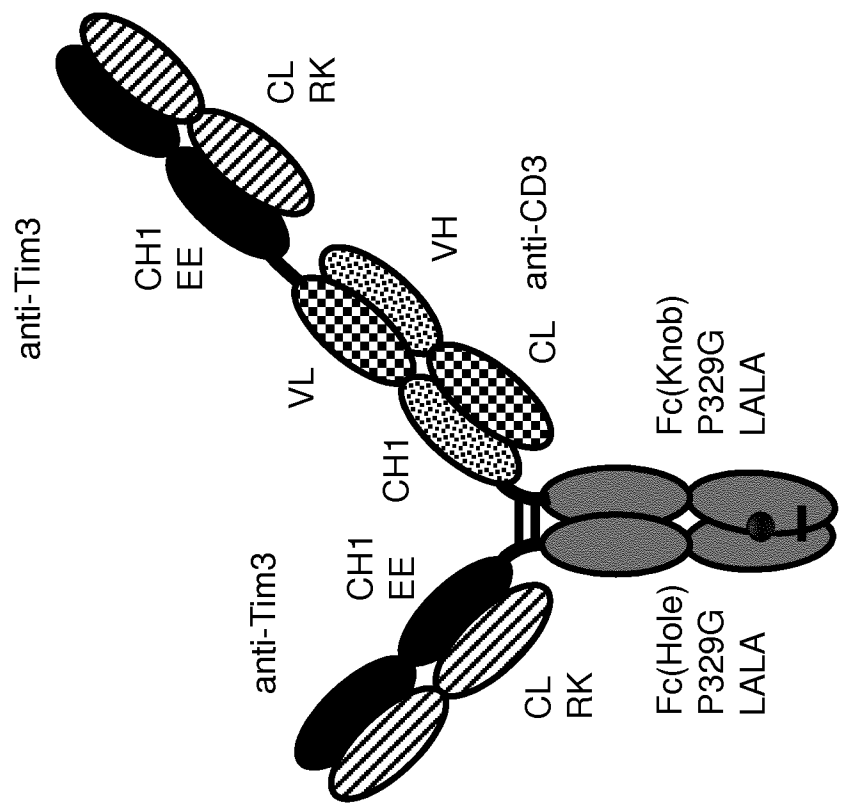
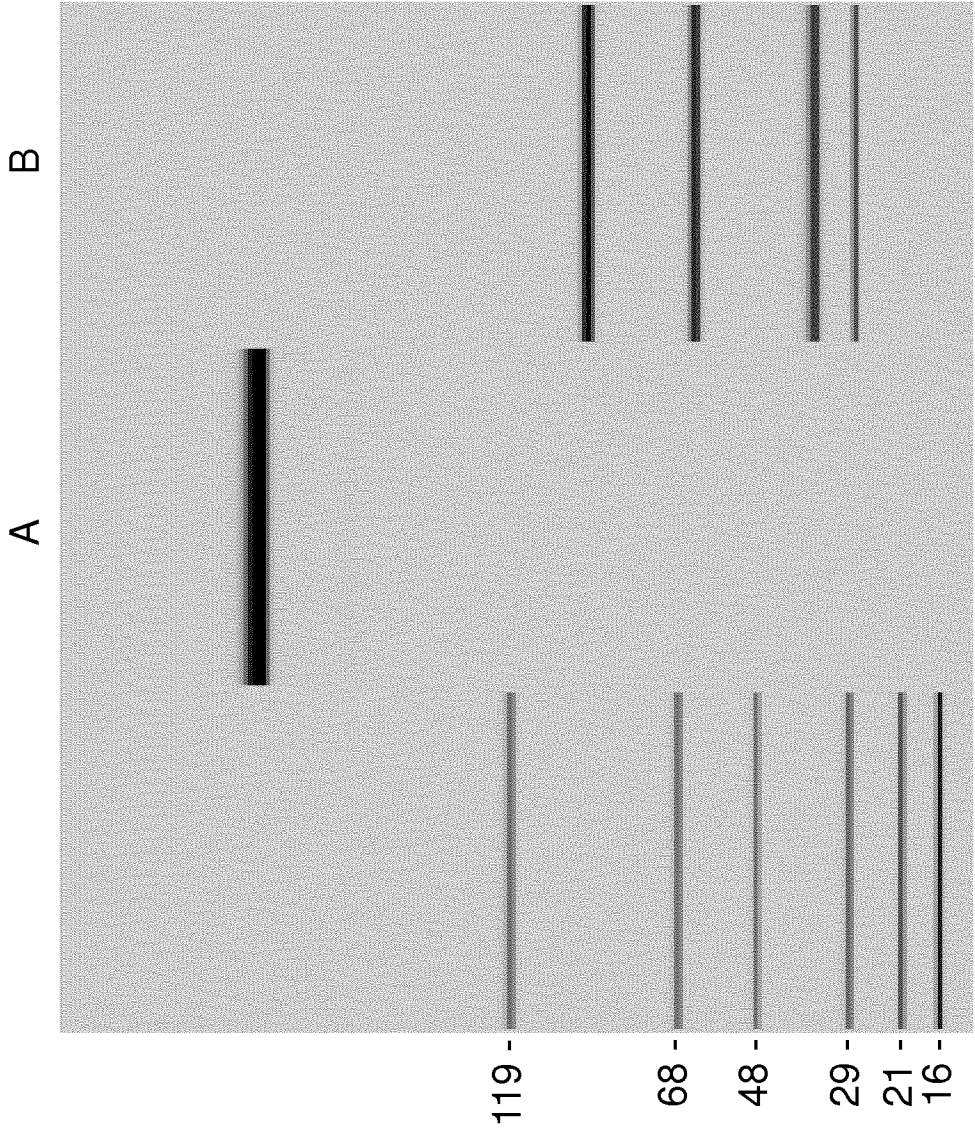


Figure 2

Figure 3



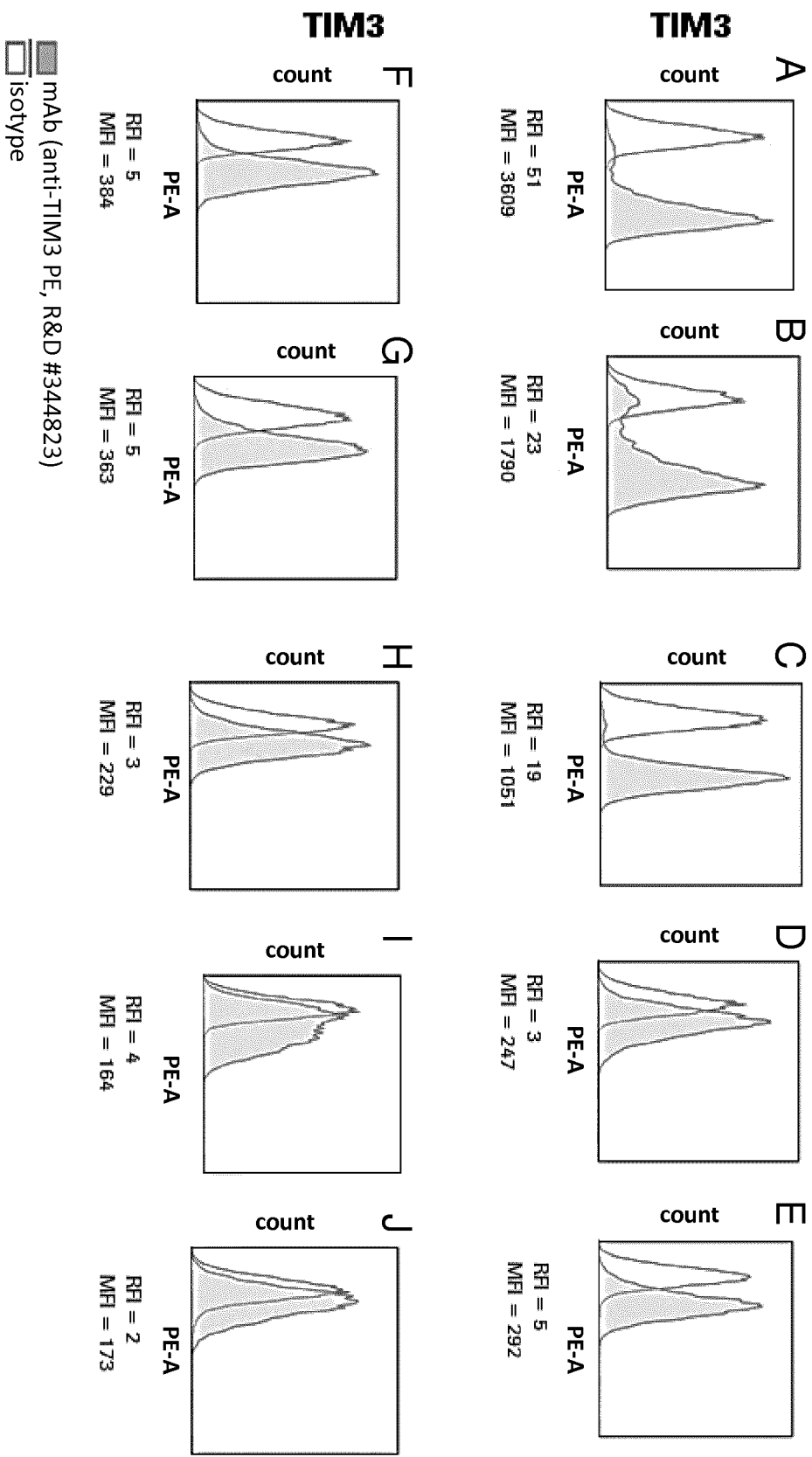


Figure 4

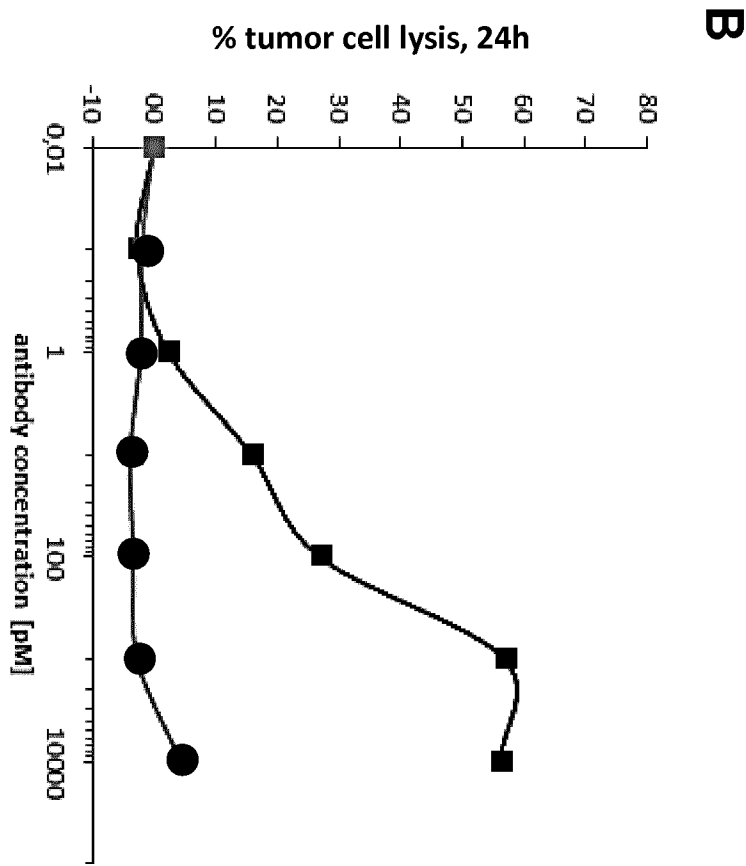
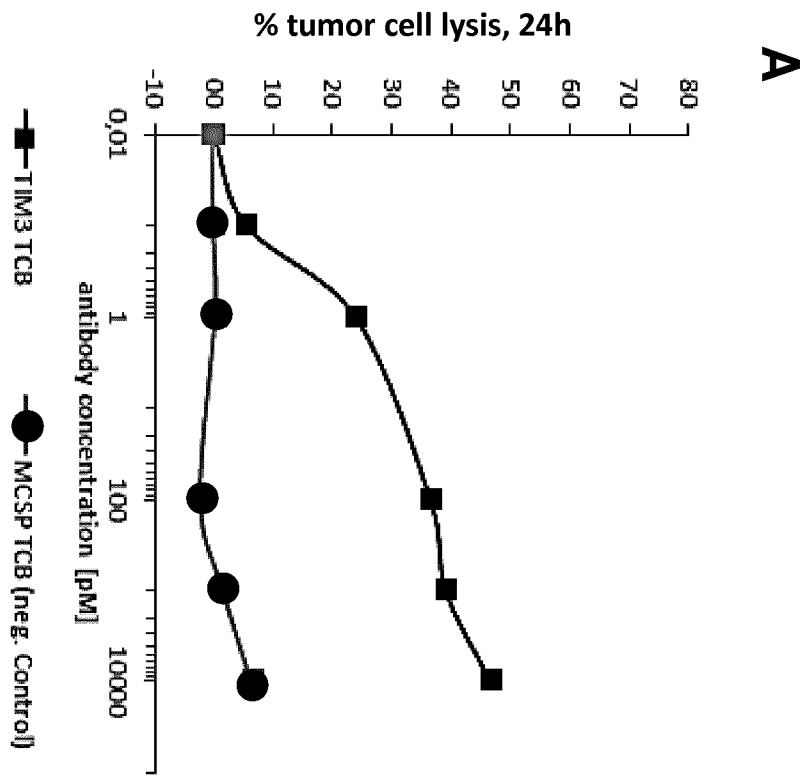


Figure 5

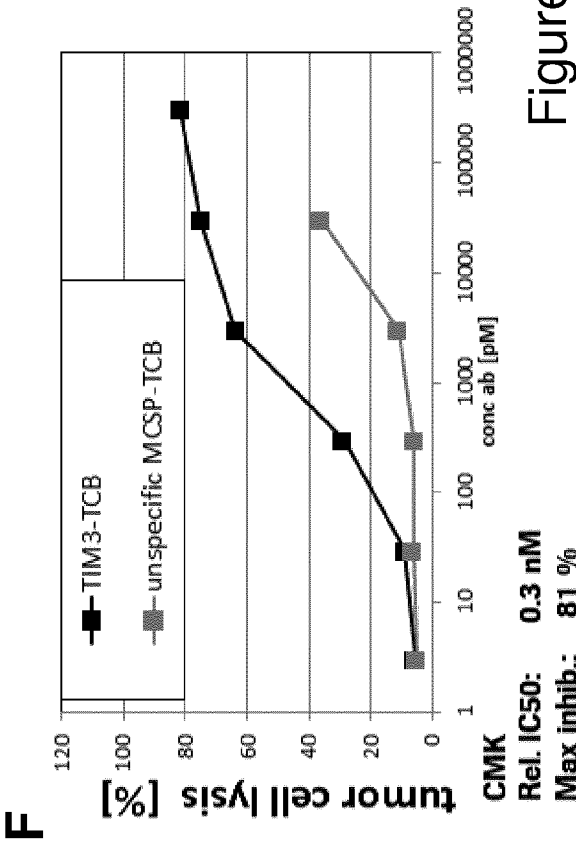
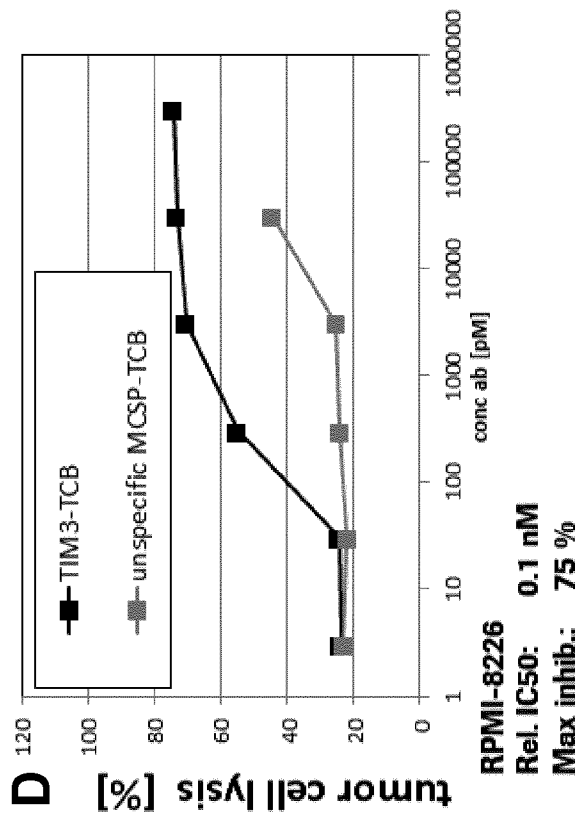
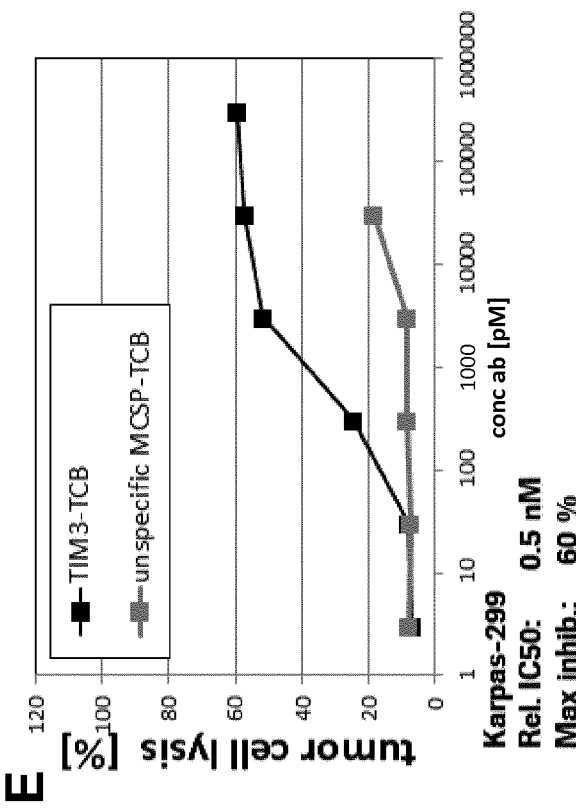
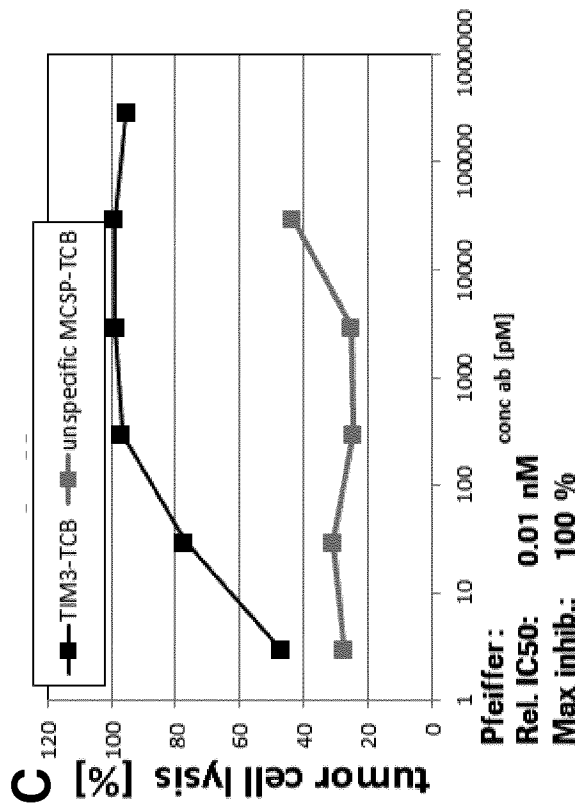


Figure 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/073176

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K16/46 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/131711 A1 (ROCHE GLYCART AG [CH]) 4 September 2014 (2014-09-04) figure 1	1-62
Y	----- K. SAKUISHI ET AL: "Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity", JOURNAL OF EXPERIMENTAL MEDICINE, vol. 207, no. 10, 27 September 2010 (2010-09-27), pages 2187-2194, XP55052551, ISSN: 0022-1007, DOI: 10.1084/jem.20100643 the whole document	1-62
A	----- WO 2015/101588 A1 (HOFFMANN LA ROCHE [CH]; HOFFMANN LA ROCHE [US]) 9 July 2015 (2015-07-09) page 53 - page 74 ----- -/--	1-62

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 December 2016

Date of mailing of the international search report

20/12/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Covone-van Hees, M

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/073176

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 63
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 63

Claim 63 does not meet the requirements of Article 6 PCT because it contains generic statements which do not define unambiguously the subject-matter for which protection is sought.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/073176

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RIDGWAY ET AL: "'KNOBS-INTO-HOLES' ENGINEERING OF ANTIBODY CH3 DOMAINS FOR HEAVY CHAIN HETERODIMERIZATION", PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 9, no. 7, 1 January 1996 (1996-01-01) , pages 617-621, XP002084766, ISSN: 0269-2139 the whole document	1-62
A	----- WO 2014/131694 A1 (ROCHE GLYCART AG [CH]) 4 September 2014 (2014-09-04) figure 1	1-62
A	----- WO 2009/080254 A1 (HOFFMANN LA ROCHE [CH]; KLEIN CHRISTIAN [DE]; SCHAEFER WOLFGANG [DE]) 2 July 2009 (2009-07-02) figure 3	1-62
A	----- CHRISTIAN KLEIN ET AL: "Progress in overcoming the chain association issue in bispecific heterodimeric IgG antibodies", MABS, vol. 4, no. 6, 1 November 2012 (2012-11-01), pages 653-663, XP55106060, ISSN: 1942-0862, DOI: 10.4161/mabs.21379 the whole document	1-62
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INTERNATIONAL SEARCH REPORT

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