

The variety of formats that are being developed shows the great potential attributed to immune cell re-direction and activation in immunotherapy. So far, developed bispecific antibodies always directly engage with the desired antigen of interest, thereby linking target cell and CTL resulting in target cell lysis. Those bispecific antibody formats face challenges related to toxicity, applicability, and producibility. Furthermore, for each single target (combination) individual molecules specific for each target need to be generated. The therapeutic utility of antibodies and their derivatives are not limited to function as T cell engagers but also find indication in modulation of inhibitory or activatory checkpoints. Exemplary, using immune checkpoint inhibiting antibodies showed durable responses in several indications (Hodie et al. N Engl J Med.; 363(8):711-23. (2010); Prieto PA, et al. Clin cancer Res.;18:2039–2047 (2012)). Furthermore, more recently, it has been shown that the activity of T cell bispecific antibodies can be further enhanced by bispecific agents activating so-called costimulatory pathways on T cells via activation of CD28 (Skokos et al., Sci Trans Med 12(525):1-14 (2020)) or 4-1BB signaling (Claus et al., Sci Trans Med 11(496), eaav5989 (2019)).

Besides current successes, therapies are limited in their flexibility to target multiple different antigens and also in their capacity to selectively exploit NK or CTL function combined in one application.

### Summary

In one aspect, the present disclosure provides an immune activating fragment crystallizable (Fc) domain binding molecule comprising

- (a) an Fc domain binding moiety that specifically binds to a target Fc domain comprising a first set of at least one amino acid substitution, and
- (b) an immune activating moiety.

In one example, the first set of at least one amino acid substitution reduce binding to an Fc receptor and/or reduce effector function.

In one example, the immune activating Fc domain binding molecule further comprising

- (c) a half-life extending Fc domain,

wherein the Fc domain binding moiety does not specifically bind to the half-life extending Fc domain.

In one example, the half-life extending Fc domain comprises a second set of at least one amino acid substitution. In one example, the second set of at least one amino acid substitution reduce binding to an Fc

In one example, the target Fc domain and/or the half-life extending Fc domain is composed of a first and a second subunit capable of stable association.

In one example, the target Fc domain and/or the half-life extending Fc domain is an IgG Fc domain, specifically an IgG<sub>1</sub> or IgG<sub>4</sub> Fc domain.

- 5 In one example, the target Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG<sub>1</sub> Fc domain.

In one example, the half-life extending Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG<sub>1</sub> Fc domain.

- 10 In one example, the first set of at least one amino acid substitution reduces binding affinity to an Fc receptor and/or effector function, and wherein the second set of at least one amino acid substitution comprises one or more amino acid substitutions at the same amino acid positions as in the first set of at least one amino acid substitution, wherein the amino acids in the second set of at least one amino acid substitution are substituted with different amino acids at the same positions compared to the first set of at least one amino acid substitution.

- 15 In one example, the second set of at least one amino acid substitution reduce binding affinity to an Fc receptor and/or effector function.

In one example, the first set of at least one amino acid substitution comprises at least one amino acid substitution at a position selected from the list consisting of 233, 234, 235, 238, 253, 265, 269, 270, 297, 310, 331, 327, 329 and 435 (numberings according to Kabat EU index).

- 20 In one example, the second set of at least one amino acid substitution comprises at least one amino acid substitution at a position selected from the list consisting of 233, 234, 235, 238, 253, 265, 269, 270, 297, 310, 331, 327, 329 and 435 (numberings according to Kabat EU index).

- 25 In one example, the first set of at least one amino acid substitution comprises the amino acid substitution P329G (numbering according to Kabat EU index) and wherein the second set of at least one amino acid substitution comprises a substitution at position P329 by an amino acid other than glycine (G) (numbering according to Kabat EU index).

In one example, the second set of at least one amino acid substitution comprises a substitution at position P329 (numbering according to Kabat EU index) by an amino acid selected from the list consisting of arginine (R), leucine (L), isoleucine (I), and alanine (A).

- 30 In one example, the second set of at least one amino acid substitution comprises a substitution at position P329 (numbering according to Kabat EU index) by arginine (R).

In another aspect, the present disclosure provides an immune activating fragment crystallizable (Fc) domain binding molecule comprising:

(a) a first light chain comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:89.

5 (b) a second light chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:70

(c) a first heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:178; and

10 (d) a second heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:179.

In another aspect, the present disclosure provides an immune activating fragment crystallizable (Fc) domain binding molecule comprising:

(a) a first light chain comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:89.

15 (b) a second light chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:68

(c) a first heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:178; and

20 (d) a second heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:179.

In another aspect, the present disclosure provides an immune activating fragment crystallizable (Fc) domain binding molecule comprising:

(a) a first light chain comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:89.

25 (b) a second light chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:180

(c) a first heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:178; and

30 (d) a second heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:179.

In another aspect, the present disclosure provides one or more isolated polynucleotide encoding the immune activating Fc domain binding molecule as described herein.

In another aspect, the present disclosure provides one or more vector, particularly expression vector, comprising the polynucleotide(s) as described herein.

- 5 In another aspect, the present disclosure provides a host cell comprising the polynucleotide(s) as described herein or the vector(s) as described herein.

In another aspect, the present disclosure provides a method of producing an immune activating fragment crystallizable (Fc) domain binding molecule, comprising the steps of a) culturing the host cell as described herein under conditions suitable for the expression of the immune activating

- 10 Fc domain binding molecule and b) recovering the immune activating Fc domain binding molecule.

In another aspect, the present disclosure provides an immune activating fragment crystallizable (Fc) domain binding molecule produced by the method as described herein.

- 15 In another aspect, the present disclosure provides a pharmaceutical composition comprising the immune activating Fc domain binding molecule as described herein and a pharmaceutically acceptable carrier.

In another aspect, the present disclosure provides the immune activating Fc domain binding molecule as described herein or the pharmaceutical composition as described herein for use as a medicament.

- 20 In another aspect, the present disclosure provides the immune activating Fc domain binding molecule as described herein or the pharmaceutical composition as described herein for use in the treatment of a disease in an individual in need thereof.

- 25 In another aspect, the present disclosure provides the immune activating Fc domain binding molecule as described herein or the pharmaceutical composition as described herein for use in the treatment of a disease in an individual in need thereof, wherein the immune activating Fc domain binding molecule is used in combination with a targeting antibody comprising the target Fc domain.

In another aspect, the present disclosure provides use of the immune activating Fc domain binding molecule as described herein in the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

In another aspect, the present disclosure provides use of an immune activating Fc domain binding molecule as described herein in the manufacture of a medicament for treating a disease in an individual, wherein

(a) a therapeutically effective amount of the medicament is to be administered to said individual  
5 in a pharmaceutically acceptable form; and

(b) a therapeutically effective amount of a composition comprising a targeting antibody comprising the target Fc domain is to be administered to said individual.

In another aspect, the present disclosure provides an *in vitro* method for inducing lysis of a cell, comprising contacting the cell with the immune activating Fc domain binding molecule as  
10 described herein and with a targeting antibody comprising the target Fc domain in the presence of a T cell, wherein the targeting antibody is capable of specific binding to an antigen on the cell.

### Claims

1. An immune activating fragment crystallizable (Fc) domain binding molecule comprising  
(a) an Fc domain binding moiety that specifically binds to a target Fc domain comprising a first set of at least one amino acid substitution, and  
5 (b) an immune activating moiety.
2. The immune activating Fc domain binding molecule of claim 1, further comprising  
(c) a half-life extending Fc,  
wherein the Fc domain binding moiety does not specifically bind to the half-life extending Fc domain.
- 10 3. The immune activating Fc domain binding molecule of claim 1 or 2, wherein the half-life extending Fc domain comprises a second set of at least one amino acid substitution.
4. The immune activating Fc domain binding molecule of any one of claims 1-3, wherein the first set of at least one amino acid substitution reduces binding affinity to an Fc receptor and/or effector function, and wherein the second set of at least one amino acid substitution comprises  
15 one or more amino acid substitutions at the same amino acid positions as in the first set of at least one amino acid substitution, wherein the amino acids in the second set of at least one amino acid substitution are substituted with different amino acids at the same positions compared to the first set of at least one amino acid substitution.
5. The immune activating Fc domain binding molecule of any one of claims 1-4, wherein the first  
20 set of at least one amino acid substitution comprises at least one amino acid substitution at a position selected from the list consisting of 233, 234, 235, 238, 253, 265, 269, 270, 297, 310, 331, 327, 329 and 435 (numberings according to Kabat EU index).
6. The immune activating Fc domain binding molecule of any one of claims 1-5, wherein the second set of at least one amino acid substitution comprises at least one amino acid substitution at  
25 a position selected from the list consisting of 233, 234, 235, 238, 253, 265, 269, 270, 297, 310, 331, 327, 329 and 435 (numberings according to Kabat EU index).
7. The immune activating Fc domain binding molecule of any one of claims 1-6, wherein the first set of at least one amino acid substitution comprises the amino acid substitution P329G (numbering according to Kabat EU index).
- 30 8. The immune activating Fc domain binding molecule of any one of claims 1-7, wherein the first set of at least one amino acid substitution comprises the amino acid substitution P329G (numbering according to Kabat EU index) and wherein the second set of at least one amino acid

substitution comprises a substitution at position P329 by an amino acid other than glycine (G) (numbering according to Kabat EU index).

9. The immune activating Fc domain binding molecule of any one of claims 1-8, wherein the second set of at least one amino acid substitution comprises a substitution at position P329 (numbering according to Kabat EU index) by an amino acid selected from the list consisting of arginine (R), leucine (L), isoleucine (I), and alanine (A).

10. The immune activating Fc domain binding molecule of any one of claims 1-9, wherein the second set of at least one amino acid substitution comprises a substitution at position P329 (numbering according to Kabat EU index) by arginine (R).

11. The immune activating Fc domain binding molecule of any one of claims 1-10, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid substitution P329G (numbering according to Kabat EU index) but not capable of specific binding to the parent non-mutated IgG1 Fc domain.

12. The immune activating Fc domain binding molecule of any one of claims 1-11, wherein the first set of at least one amino acid substitution comprises at least one amino acid substitution at a position selected from the group of L234, L235 (Kabat EU index numbering).

13. The immune activating Fc domain binding molecule of any one of claims 1-12, wherein the second set of at least one amino acid substitution comprises at least one amino acid substitution at a position selected from the group of L234, L235 (Kabat EU index numbering).

14. The immune activating Fc domain binding molecule of any one of claims 1-10, wherein the target 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).

15. The immune activating Fc domain binding molecule of any one of claims 1-14, wherein the half-life extending 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 P329X (Kabat EU index numbering), wherein X is an amino acid other than glycine (G).

16. The immune activating Fc domain binding molecule of any one of claims 1-15, wherein the Fc domain binding moiety and/or the immune activating moiety is a Fab molecule.

17. The immune activating Fc domain binding molecule of any one of claims 1-16, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the

amino acid substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises:

(i) a heavy chain variable region (VH) comprising

(a) the heavy chain complementarity-determining region (CDR H) 1 amino acid sequence RYWMN (SEQ ID NO:1);

(b) a CDR H2 amino acid sequence selected from the group consisting of EITPDSSTINYTPSLKD (SEQ ID NO:2), EITPDSSTINYTPSLKG (SEQ ID NO:11) and EITPDSSTINYAPSLKG (SEQ ID NO:16); and

(c) the CDR H3 amino acid sequence PYDYGAWFAS (SEQ ID NO:3); and

(ii) a light chain variable region (VL) comprising

(d) the light chain complementary-determining region (CDR L) 1 amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO:4);

(e) the CDR L2 amino acid sequence GTNKRAP (SEQ ID NO:5); and

(f) the CDR L3 amino acid sequence ALWYSNHWV (SEQ ID NO:6).

18. The immune activating Fc domain binding molecule of claim 17, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises:

(i) a heavy chain variable region (VH) comprising

(a) the heavy chain complementarity-determining region (CDR H) 1 amino acid sequence RYWMN (SEQ ID NO:1);

(b) the CDR H2 amino acid sequence EITPDSSTINYTPSLKD (SEQ ID NO:2); and

(c) the CDR H3 amino acid sequence PYDYGAWFAS (SEQ ID NO:3); and

(ii) a light chain variable region (VL) comprising

(d) the light chain complementary-determining region (CDR L) 1 amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO:4);

(e) the CDR L2 amino acid sequence GTNKRAP (SEQ ID NO:5); and

(f) the CDR L3 amino acid sequence ALWYSNHWV (SEQ ID NO:6).

19. The immune activating Fc domain binding molecule of claim 17, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises:

(i) a heavy chain variable region (VH) comprising

(a) the heavy chain complementarity-determining region (CDR H) 1 amino acid sequence RYWMN (SEQ ID NO:1);

(b) the CDR H2 amino acid sequence EITPDSSTINYTPSLKG (SEQ ID NO:11); and

(c) the CDR H3 amino acid sequence PYDYGAWFAS (SEQ ID NO:3); and

5 (ii) a light chain variable region (VL) comprising

(d) the light chain complementary-determining region (CDR L) 1 amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO:4);

(e) the CDR L2 amino acid sequence GTNKRAP (SEQ ID NO:5); and

(f) the CDR L3 amino acid sequence ALWYSNHWV (SEQ ID NO:6).

10 20. The immune activating Fc domain binding molecule of claim 17, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises:

(i) a heavy chain variable region (VH) comprising

15 (a) the heavy chain complementarity-determining region (CDR H) 1 amino acid sequence RYWMN (SEQ ID NO:1);

(b) the CDR H2 amino acid sequence EITPDSSTINYAPSLKG (SEQ ID NO:16); and

(c) the CDR H3 amino acid sequence PYDYGAWFAS (SEQ ID NO:3); and

(ii) a light chain variable region (VL) comprising

20 (d) the light chain complementary-determining region (CDR L) 1 amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO:4);

(e) the CDR L2 amino acid sequence GTNKRAP (SEQ ID NO:5); and

(f) the CDR L3 amino acid sequence ALWYSNHWV (SEQ ID NO:6).

21. The immune activating Fc domain binding molecule of any one of claims 1-20, wherein the  
25 Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:12, SEQ ID NO:17 and SEQ ID NO:19, and a light chain variable  
30 region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:13.

22. The immune activating Fc domain binding molecule of claim 21, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid

substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises

(i) a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 7 and a light chain variable region sequence that is at least about

5 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 8,

(ii) a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 12 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 13,

(iii) a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or  
10 100% identical to SEQ ID NO: 17 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 13, or

(iv) a heavy chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 19 and a light chain variable region sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 13.

15 23. The immune activating Fc domain binding molecule of claim 22, wherein the Fc domain binding moiety is capable of specific binding to an IgG1 Fc domain comprising the amino acid substitution P329G (numbering according to Kabat EU index), wherein the Fc domain binding moiety comprises the heavy chain variable region sequence of SEQ ID NO: 19 and the light chain variable of SEQ ID NO: 13.

20 24. The immune activating Fc domain binding molecule of any one of claims 1-23, wherein the Fc domain binding moiety comprises a first Fab molecule and the immune activating moiety comprises a second Fab molecule.

25. The immune activating Fc domain binding molecule according to claim 24, further comprising

25 d) a third Fab molecule which specifically binds to the target Fc domain comprising the first set of at least one amino acid substitution.

26. The immune activating Fc domain binding molecule according to claim 25 wherein the third Fab molecule is identical to the first Fab molecule.

27. The immune activating Fc domain binding molecule of any one of claims 1-26, wherein the  
30 immune activating moiety is capable of specific binding to an activating T cell antigen.

28. The immune activating Fc domain binding molecule of claim 27, wherein the activating T cell antigen is CD3.

29. The immune activating Fc domain binding molecule of claim 27 or 28, wherein the activating T cell antigen is CD3 epsilon.

30. The immune activating Fc domain binding molecule of any one of claims 27-29, wherein the immune activating moiety specifically binds to an activating T cell antigen, particularly CD3, more particularly CD3 epsilon.

31. The immune activating Fc domain binding molecule of any one of claims 27-30, wherein the immune activating moiety is a Fab molecule.

32. The immune activating Fc domain binding molecule molecule of claim 31, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 35, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain CDR 3 of SEQ ID NO: 43, the light chain CDR 1 of SEQ ID NO: 53, the light chain CDR 2 of SEQ ID NO: 54 and the light chain CDR 3 of SEQ ID NO: 55.

33. The immune activating Fc domain binding molecule molecule of claim 31 or 32, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen 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: 49 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: 56.

34. The immune activating Fc domain binding molecule molecule of claim 31, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 35, the heavy chain CDR 2 of SEQ ID NO: 33, the heavy chain CDR 3 of SEQ ID NO: 176, the light chain CDR 1 of SEQ ID NO: 53, the light chain CDR 2 of SEQ ID NO: 54 and the light chain CDR 3 of SEQ ID NO: 55.

35. The immune activating Fc domain binding molecule molecule of claim 31 or 34, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen 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: 177 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: 56.

36. The immune activating Fc domain binding molecule molecule of claim 31, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 34, the heavy chain CDR 2 of SEQ ID NO: 37, the heavy chain  
5 CDR 3 of SEQ ID NO: 41, the light chain CDR 1 of SEQ ID NO: 53, the light chain CDR 2 of SEQ ID NO: 54 and the light chain CDR 3 of SEQ ID NO: 55.

37. The immune activating Fc domain binding molecule molecule of claim 31 or 36, wherein the activating T cell antigen is CD3, particularly CD3 epsilon, and the Fab molecule which specifically binds to the activating T cell antigen comprises a heavy chain variable region comprising an amino  
10 acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 47 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: 56.

38. An immune activating fragment crystallizable (Fc) domain binding molecule comprising:

15 (a) a first light chain comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:89.

(b) a second light chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:70

(c) a first heavy chain comprising the amino acid sequence that is at least about 95%, 96%,  
20 97%, 98%, 99% or 100% identical to SEQ ID NO:178; and

(d) a second heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:179.

39. An immune activating fragment crystallizable (Fc) domain binding molecule comprising:

(a) a first light chain comprising an amino acid sequence that is at least about 95%, 96%,  
25 97%, 98%, 99% or 100% identical to SEQ ID NO:89.

(b) a second light chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:68

(c) a first heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:178; and

30 (d) a second heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:179.

40. An immune activating fragment crystallizable (Fc) domain binding molecule comprising:

(a) a first light chain comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:89.

(b) a second light chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:180

5 (c) a first heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:178; and

(d) a second heavy chain comprising the amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:179.

10 41. The immune activating Fc domain binding molecule of any one of claims 1-26, wherein the immune activating moiety is capable of specific binding to a costimulatory T cell antigen.

42. The immune activating Fc domain binding molecule of claim 41, wherein the costimulatory T cell antigen is CD28.

43. The immune activating Fc domain binding molecule of claim 41 or 42, wherein the immune activating moiety specifically binds to a costimulatory T cell antigen.

15 44. The immune activating Fc domain binding molecule of any one of claims 41-43, wherein the immune activating moiety specifically binds to CD28.

45. The immune activating Fc domain binding molecule of any one of claims 41-44, wherein the immune activating moiety is a Fab molecule.

20 46. The immune activating Fc domain binding molecule molecule of claim 45, wherein the Fab molecule which specifically binds to CD28 comprises the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 94, the heavy chain CDR 2 of SEQ ID NO: 95, the heavy chain CDR 3 of SEQ ID NO: 96, the light chain CDR 1 of SEQ ID NO: 97, the light chain CDR 2 of SEQ ID NO: 98 and the light chain CDR 3 of SEQ ID NO: 99; or the heavy chain complementarity determining region (CDR) 1 of SEQ ID NO: 94, the heavy chain CDR 2 of SEQ ID NO: 95, the heavy chain CDR 3 of SEQ ID NO: 102, the light chain CDR 1 of SEQ ID NO: 103, the light chain CDR 2 of SEQ ID NO: 98 and the light chain CDR 3 of SEQ ID NO: 99

25 47. The immune activating Fc domain binding molecule molecule of claims 45 or 46, wherein the Fab molecule which specifically binds CD28 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: 100 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: 101; or 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: 104 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: 105.

48. The immune activating Fc domain binding molecule of any one of claims 1-26, wherein the immune activating moiety is capable of specific binding to a costimulatory T cell antigen.

5 49. The immune activating Fc domain binding molecule of claim 48, wherein the costimulatory T cell antigen is 4-1BB.

50. The immune activating Fc domain binding molecule of claim 48 or 49, wherein the immune activating moiety specifically binds to a costimulatory T cell antigen.

10 51. The immune activating Fc domain binding molecule of any one of claims 48-50, wherein the immune activating moiety specifically binds to 4-1BB.

52. The immune activating Fc domain binding molecule of any one of claims 48-51, wherein the immune activating moiety is a Fab molecule.

53. The immune activating Fc domain binding molecule molecule of claim 52, wherein Fab molecule which specifically binds to 4-1BB comprises the heavy chain complementarity  
15 determining region (CDR) 1 of SEQ ID NO: 133, the heavy chain CDR 2 of SEQ ID NO: 134, the heavy chain CDR 3 of SEQ ID NO: 135, the light chain CDR 1 of SEQ ID NO: 136, the light chain CDR 2 of SEQ ID NO: 137 and the light chain CDR 3 of SEQ ID NO: 138.

54. The immune activating Fc domain binding molecule molecule of any one of claims 52 or 53, wherein the Fab molecule which specifically binds to 4-1BB comprises a heavy chain variable  
20 region comprising i) 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: 139 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: 140.

55. The immune activating fragment crystallizable (Fc) domain binding molecule of any one of  
25 claims 1-23, wherein the immune activating moiety is a cytokine.

56. The immune activating fragment crystallizable (Fc) domain binding molecule of claim 55, wherein the cytokine is selected from the group consisting of IL2, IL7, IL15, IL18, IFN $\alpha$  and IFN $\gamma$ .

57. The immune activating fragment crystallizable (Fc) domain binding molecule of claim 55 or 56, wherein the immune activating moiety is a mutant interleukin-2 (IL-2) polypeptide.

30 58. The immune activating fragment crystallizable (Fc) domain binding molecule of claim 57, wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO:166).

59. The immune activating fragment crystallizable (Fc) domain binding molecule of claim 58, wherein the mutant IL-2 polypeptide further comprises the amino acid substitution T3A and/or the amino acid substitution C125A.

60. The immune activating fragment crystallizable (Fc) domain binding molecule of any one of claims 57-59, wherein the mutant IL-2 polypeptide comprises 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:167, wherein the mutant IL-2 polypeptide shows reduced affinity to the high-affinity IL-2 receptor and substantially similar affinity to the intermediateaffinity IL-2 receptor, each compared to a wild-type IL-2 polypeptide.

61. The immune activating fragment crystallizable (Fc) domain binding molecule of any one of claims 57-60, wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO:167.

62. The immune activating Fc domain binding molecule of any one of claims 1-23, wherein the immune activating moiety comprises three ectodomains of 4-1BBL or a fragment thereof.

63. The immune activating Fc domain binding molecule of any one of claims 1-23, wherein the immune activating moiety comprises a first and a second polypeptide, wherein the first polypeptide contains a first heavy chain constant (CH1) or a light chain constant (CL) domain and the second polypeptide contains a CL or CH1 domain, respectively, wherein the second polypeptide is linked to the first polypeptide by a disulfide bond between the CH1 and CL domain, and wherein the first polypeptide comprises two ectodomains of 4-1BBL or a fragment thereof that are connected to each other and to the CH1 or CL domain by a peptide linker and wherein the second polypeptide comprises one ectodomain of said 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide.

64. The immune activating Fc domain binding molecule of claim 62 or 63, wherein the ectodomain of 4-1BBL or a fragment thereof comprises the amino acid sequence selected from the group consisting of SEQ ID NO:117, SEQ ID NO: 118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO: 122, SEQ ID NO:123 and SEQ ID NO:124, particularly the amino acid sequence of SEQ ID NO:117 or SEQ ID NO:121.

65. The immune activating Fc domain binding molecule of any one of claims 62-64, wherein the immune activating moiety comprises a first and a second polypeptide that are linked to each other by a disulfide bond, wherein the antigen binding molecule is characterized in that the first polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127 and SEQ ID NO:128 and in that the second

polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:117, SEQ ID NO:121, SEQ ID NO:119 and SEQ ID NO:120.

5 66. The immune activating Fc domain binding molecule of any one of claims 1-23 or 62-65, wherein the molecule comprises a first heavy chain and a first light chain comprising the Fc domain binding moiety, in particular a Fab molecule capable of specific binding to the target Fc domain, and a second heavy chain and a second light chain comprising the immune activating moiety, wherein the second heavy chain comprises the first polypeptide comprising two ectodomains of 4-1BBL or a fragment thereof that are connected to each other and to the CH1 or CL domain by a peptide and the second light chain comprises the second polypeptide comprising  
10 one ectodomain of said 4-1BBL or a fragment thereof connected via a peptide linker to the CL or CH1 domain of said polypeptide, respectively.

67. The immune activating Fc domain binding molecule of any one of claims 63-66, wherein the first peptide comprising two ectodomains of 4-1BBL or a fragment thereof connected to each other by a first peptide linker is fused at its C-terminus by a second peptide linker to a CL domain that  
15 is part of a heavy chain, and the second peptide comprising one ectodomain of said 4-1BBL or a fragment thereof is fused at its C-terminus by a third peptide linker to a CH1 domain that is part of a light chain.

68. The immune activating Fc domain binding molecule of any one of claims 1-23, wherein the immune activating moiety is capable of specific binding to an Fc receptor.

20 69. The immune activating Fc domain binding molecule of claim 68, wherein the Fc receptor is a Fc gamma receptor, in particular the FcγRIIIa receptor.

70. The immune activating Fc domain binding molecule of claim 68 or 69, wherein the Fc receptor is CD16.

25 71. One or more isolated polynucleotide encoding the immune activating Fc domain binding molecule of any one of claims 1 to 70.

72. One or more vector, particularly expression vector, comprising the polynucleotide(s) of claim 71.

73. A host cell comprising the polynucleotide(s) of claim 71 or the vector(s) of claim 72.

30 74. A method of producing an immune activating fragment crystallizable (Fc) domain binding molecule, comprising the steps of a) culturing the host cell of claim 73 under conditions suitable for the expression of the immune activating Fc domain binding molecule and b) recovering the immune activating Fc domain binding molecule.

75. An immune activating fragment crystallizable (Fc) domain binding molecule produced by the method of claim 74.

76. A pharmaceutical composition comprising the immune activating Fc domain binding molecule of any one of claims 1 to 70 and a pharmaceutically acceptable carrier.

5 77. The immune activating Fc domain binding molecule of any one of claims 1 to 70 or the pharmaceutical composition of claim 76 for use as a medicament.

78. The immune activating Fc domain binding molecule of any one of claims 1 to 70 or the pharmaceutical composition of claim 76 for use in the treatment of a disease in an individual in need thereof.

10 79. The immune activating Fc domain binding molecule or the pharmaceutical composition of for use according to claim 78, wherein the disease is cancer.

80. The immune activating Fc domain binding molecule of any one of claims 1 to 70 or the pharmaceutical composition of claim 76 for use in the treatment of a disease in an individual in need thereof, wherein the immune activating Fc domain binding molecule is used in combination

15 with a targeting antibody comprising the target Fc domain.

81. The immune activating Fc domain binding molecule or the pharmaceutical composition of for use according to claim 80, wherein the disease is cancer.

82. The immune activating Fc domain binding molecule or the pharmaceutical composition for use of claim 80 or 81, wherein the targeting antibody is capable of specific binding to a target  
20 antigen, in particular a target antigen on a cancer cell.

83. Use of the immune activating Fc domain binding molecule of any one of claims 1 to 70 in the manufacture of a medicament for the treatment of a disease in an individual in need thereof.

84. The use of claim 83, wherein said disease is cancer.

85. Use of an immune activating Fc domain binding molecule of any one of claims 1 to 70 in the  
25 manufacture of a medicament for treating a disease in an individual, wherein

(a) a therapeutically effective amount of the medicament is to be administered to said individual in a pharmaceutically acceptable form; and

(b) a therapeutically effective amount of a composition comprising a targeting antibody comprising the target Fc domain is to be administered to said individual.

30 86. The use of claim 84, wherein the disease is cancer.

87. The use of claim 84 or 86, wherein the targeting antibody is capable of specific binding to a target antigen, in particular a target antigen on a cancer cell.

88. The use of any one of claims 85-87 wherein immune activating Fc domain binding molecules is to be administered before, at the same time, or after the antibody comprising the target Fc domain.

89. An *in vitro* method for inducing lysis of a cell, comprising contacting the cell with the immune activating Fc domain binding molecule of any one of claims 1-70 and with a targeting antibody

5 comprising the target Fc domain in the presence of a T cell, wherein the targeting antibody is capable of specific binding to an antigen on the cell.