binders disclosed therein, use of a crossover-Fab based bispecific format results in improved yield and less aggregates and side-products during production. In addition, scFv- and scFab-based DR5-FAP targeting bispecific antibodies demonstrated the tendency to form aggregates and therefore bear a higher riskto non-specifically crosslinkDR5 even in absence of FAP. Hence these formats have the disadvantage of potentially inducing apoptosis in non-target cells (i.e. healthy cells).

The DR5 and FAP binding moieties of the novel bispecific antibodies provided herein exhibit superior in vivo efficacy compared to conventional DR5 antibodies. The preferred bispecific antibodies of the present invention bind with a high affinity to FAP on the tumor stroma and with a lower affinity to DR5 on the tumor cell. Moreover, the DR5 and FAP targeting bispecific antibodies provided herein do not crossreact with closely related proteins DR4, DcR1, DcR2 (closely related to DR5) and DPPIV (closely related to FAP). In addition it is now for the first time possible to provide a DR5-FAP bispecific antibody in various bispecific formats with no limitation as to the number of valencies of each binding specifity.

To summarize, the novel bispecific DR5-FAP antibodies provided therein are highly specific and potent: They selectively induce apoptosis by DR5 hyperclustering in tumor cells in a FAP dependent manner, with low binding to normal cells.

SUMMARY

The present ~~invention~~ application relates to bispecific antibodies combining a Death Receptor 5 (DR5) targeting antigen binding site with a second antigen binding site that targets Fibroblast Activation Protein (FAP). By that the death receptors become cross linked and apoptosis of the targeted tumor cell is induced. The advantage of these bispecific death receptor agonistic antibodies over conventional death receptor targeting antibodies is the specificity of induction of apoptosis only at the site where FAP is expressed as well as the higher potency of these bispecific antibodies due to the induction of DR5 hyperclustering. In addition, novel antibodies binding to DR5 are provided. As outlined above the inventors of the present application ~~invention~~ developed novel DR5 binding moieties with superior properties compared to known DR5 binders that are incorporated into novel and advantageous DR5-FAP bispecific antibodies.

~~In one embodiment, t~~The application ~~invention~~ provides a bispecific antibody that binds to death receptor 5 (DR5) and Fibroblast Activation Protein (FAP), comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 consisting of SEQ ID NO.:1, SEQ ID NO.:17 and SEQ ID NO.:75;

(b) a heavy chain CDR2 of SEQ ID NO.:2, SEQ ID NO.:18, SEQ ID NO.:25 and SEQ ID NO.:83;

(c) a heavy chain CDR3 of SEQ ID NO.:3, SEQ ID NO.:19, SEQ ID NO.:84, SEQ ID NO.:96, SEQ ID NO.:98, SEQ ID NO.:104 and SEQ ID NO.:108;

(d) a light chain CDR1 of SEQ ID NO.:4, SEQ ID NO.:20, SEQ ID NO.:27 and SEQ ID NO.:86;

(e) a light chain CDR2 of SEQ ID NO.:5, SEQ ID NO.:21 and SEQ ID NO.:28; and

(f) a light chain CDR3 of SEQ ID NO.:6, SEQ ID NO.:22, SEQ ID NO.:87, SEQ ID NO.:99, SEQ ID NO.:105, SEQ ID NO.:109 and SEQ ID NO.:97;

and at least one antigen binding site specific for FAP, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:9 and SEQ ID NO.:33;

(b) a heavy chain CDR2 of SEQ ID NO.:10 and SEQ ID NO.:34;

(c) a heavy chain CDR3 of SEQ ID NO.:11 and SEQ ID NO.:35;

(d) a light chain CDR1 of SEQ ID NO.:12 and SEQ ID NO.:36;

(e) a light chain CDR2 of SEQ ID NO.:13 and SEQ ID NO.:37;

(f) a light chain CDR3 of SEQ ID NO.:14 and SEQ ID NO.:38.

In one aspect, the present application provides a bispecific antibody that binds to death receptor 5 (DR5) and Fibroblast Activation Protein (FAP), comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain complementarity determining region 1 (CDR1) of SEQ ID NO.:1;

(b) a heavy chain complementarity determining region 2 (CDR2) of SEQ ID NO.:2;

(c) a heavy chain complementarity determining region 3 (CDR3) selected from the group of SEQ ID NO.:3, SEQ ID NO.:96, SEQ ID NO.:98; SEQ ID NO.:104 and SEQ ID NO.:108;

(d) a light chain CDR1 of SEQ ID NO.:4;

(e) a light chain CDR2 of SEQ ID NO.:5; and

(f) a light chain CDR3 selected from the group of SEQ ID NO.:6, SEQ ID NO.:99, SEQ ID NO.:105, SEQ ID NO.:109 and SEQ ID NO.:97;

and at least one antigen binding site specific for FAP, comprising

(a) a heavy chain CDR1 selected from the group of SEQ ID NO.:9 and SEQ ID NO.:33;

(b) a heavy chain CDR2 selected from the group of SEQ ID NO.:10 and SEQ ID NO.:34;

(c) a heavy chain CDR3 selected from the group of SEQ ID NO.:11 and SEQ ID NO.:35;

(d) a light chain CDR1 selected from the group of SEQ ID NO.:12 and SEQ ID NO.:36;

(e) a light chain CDR2 selected from the group of SEQ ID NO.:13 and SEQ ID NO.:37;

(f) a light chain CDR3 selected from the group of SEQ ID NO.:14 and SEQ ID NO.:38.

In one embodiment, the application ~~invention~~ provides a bispecific antibody wherein the antigen binding site specific for DR5 comprises

(a) a heavy chain CDR1 of SEQ ID NO.:1;

(b) a heavy chain CDR2 of SEQ ID NO.:2;

(c) a heavy chain CDR3 of SEQ ID NO.:3;

(d) a light chain CDR1 of SEQ ID NO.:4;

(e) a light chain CDR2 of SEQ ID NO.:5;

(f) a light chain CDR3 of SEQ ID NO.:6

and the antigen binding site specific for FAP comprises

(a) a heavy chain CDR1 of SEQ ID NO.:9;

(b) a heavy chain CDR2 of SEQ ID NO.:10;

(c) a heavy chain CDR3 of SEQ ID NO.:11;

(d) a light chain CDR1 of SEQ ID NO.:12;

(e) a light chain CDR2 of SEQ ID NO.:13;

comprises three amino acid substitutions that abolish binding to an activating or inhibitory Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G.

In one embodiment a bispecific antibody is provided wherein the Fc part of the first heavy chain comprises a first dimerization module and the Fc part of the second heavy chain comprises a second dimerization module allowing a heterodimerization of the two heavy chains of the antibody. In one embodiment the first dimerization module comprises knobs and the second dimerization module comprises holes according to the knobs into holes strategy.

The application also provides ~~In a further embodiment~~ an antibody that specifically binds to DR5 ~~is provided~~, comprising

(a) a heavy chain complementarity determining region 1 (CDR1) selected from the group consisting of SEQ ID NO.:1, SEQ ID NO.:17 and SEQ ID NO.:75;

(b) a heavy chain complementarity determining region 2(CDR2) selected from the group of SEQ ID NO.:2, SEQ ID NO.:18, SEQ ID NO.:25 and SEQ ID NO.:83;

(c) a heavy chain complementarity determining region 3 (CDR3) selected from the group of SEQ ID NO.:3, SEQ ID NO.:19, SEQ ID NO.:84, SEQ ID NO.:96, SEQ ID NO.:98, SEQ ID NO.:104 and SEQ ID NO.:108;

(d) a light chain CDR1 selected from the group of SEQ ID NO.:4, SEQ ID NO.:20, SEQ ID NO.:27 and SEQ ID NO.:86;

(e) a light chain CDR2 selected from the group of SEQ ID NO.:5, SEQ ID NO.:21 and SEQ ID NO.:28; and

(f) a light chain CDR3 selected from the group of SEQ ID NO.:6, SEQ ID NO.:22, SEQ ID NO.:87, SEQ ID NO.:99, SEQ ID NO.:105, SEQ ID NO.:109 and SEQ ID NO.:97~~;~~.

In another aspect, the present application provides an antibody that specifically binds to DR5, comprising

(a) a heavy chain complementarity determining region 1 (CDR1) of SEQ ID NO.:1;
(b) a heavy chain complementarity determining region 2 (CDR2) of SEQ ID NO.:2;
(c) a heavy chain complementarity determining region 3 (CDR3) selected from the group of SEQ ID NO.:3, SEQ ID NO.:96, SEQ ID NO.:98, SEQ ID NO.:104 and SEQ ID NO.:108;

(d) a light chain CDR1 of SEQ ID NO.:4;

(e) a light chain CDR2 of SEQ ID NO.:5; and

(f) a light chain CDR3 selected from the group of SEQ ID NO.:6, SEQ ID NO.:99, SEQ ID NO.:105, SEQ ID NO.:109 and SEQ ID NO.:97.

In a further embodiment an antibody that specifically binds to DR5 is provided, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:1;

(b) a heavy chain CDR2 of SEQ ID NO.:2;

(c) a heavy chain CDR3 of SEQ ID NO.:3;

(d) a light chain CDR1 of SEQ ID NO.:4;

(e) a light chain CDR2 of SEQ ID NO.:5;

(f) a light chain CDR3 of SEQ ID NO.:6

In a further embodiment an antibody that specifically binds to DR5 is provided, comprising a variable heavy chain and a variable light chain comprising an amino acid sequence selected from the group of: SEQ ID NO.:7 and SEQ ID NO.:8; SEQ ID NO.:23 and SEQ ID NO.:24; SEQ ID NO.:26 and SEQ ID NO.:24; SEQ ID NO.:23 and SEQ ID NO.:29; SEQ ID NO.:23 and SEQ ID NO.:30; SEQ ID NO.:26 and SEQ ID NO.:31; SEQ ID NO.:26 and SEQ ID NO.:32; SEQ ID NO.:26 and SEQ ID NO.:30; SEQ ID NO.:23 and SEQ ID NO.:31; SEQ ID NO.:82 and SEQ ID NO.:85; SEQ ID NO.:100 and SEQ ID NO.:101; SEQ ID NO.:102 and SEQ ID NO.:103; SEQ ID NO.:106 and SEQ ID NO.:107; SEQ ID NO.:94 and SEQ ID NO.:95;

In a further embodiment an antibody that specifically binds to DR5 is provided comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:7 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:8.

In ~~a second object~~ another aspect, the present application ~~invention~~ relates to a pharmaceutical composition comprising a bispecific antibody or the antibody that specifically binds to DR5 of the present application~~invention~~.

In another aspect, ~~a third object~~ the present application ~~invention~~ relates to a bispecific antibody targeting DR5 and FAP or an antibody that specifically binds to DR5 of the present application ~~invention~~ for the treatment of cancer. In one preferred embodiment said cancer is pancreatic cancer or colorectal carcinoma. In another embodiment, use of the bispecific antibody or an antibody that specifically binds to DR5 as a medicament is provided. Preferably said use is for the treatment of cancer, preferably pancreatic cancer or colorectal carcinoma. In another aspect, the present application relates to use of the bispecific antibody targeting DR5 and FAP or an antibody that specifically binds to DR5 of the present application in the manufacture of a medicament.

~~In further objects t~~The present application ~~invention~~ also relates to a nucleic acid sequence comprising a sequence encoding a heavy chain of a bispecific antibody or an antibody that specifically binds to DR5 of the present application~~invention~~. In another aspect, the present application relates to a nucleic acid sequence comprising a sequence encoding a heavy chain of an antibody that specifically binds to DR5 of the present application. The present application also relates to~~,~~ a nucleic acid sequence comprising a sequence encoding a light chain of a bispecific antibody or an antibody that specifically binds to DR5 of the present application~~invention~~.~~,~~ In another aspect, the present application relates to a nucleic acid sequence comprising a sequence encoding a light chain of an antibody that specifically binds to DR5 of the present application. In another aspect, the present application relates to an expression vector comprising a nucleic acid sequence of the present application. ~~invention~~ In another aspect, the present application relates to ~~and to~~ a prokaryotic or eukaryotic host cell comprising a vector of the present application~~invention~~. In another aspect, the present application relates to ~~In addition~~ a

method of producing an antibody comprising culturing the host cell so that the antibody is produced ~~is provided~~.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: FACS binding analysis of two different cell lines (SW872 and GM05389) for expression levels of human fibroblast activation protein (FAP) (A). The fluorescence intensity

Claims

1. A bispecific antibody that binds to death receptor 5 (DR5) and Fibroblast Activation Protein (FAP), comprising at least one antigen binding site specific for DR5, comprising
(a) a heavy chain complementarity determining region 1 (CDR1) ~~selected from the group consisting~~ of SEQ ID NO.:1~~, SEQ ID NO.:17 and SEQ ID NO.:75~~;
(b) a heavy chain complementarity determining region 2 (CDR2) ~~selected from the group~~ of SEQ ID NO.:2~~, SEQ ID NO.:18, SEQ ID NO.:25 and SEQ ID NO.:83~~;
(c) a heavy chain complementarity determining region 3 (CDR3) selected from the group of SEQ ID NO.:3, ~~SEQ ID NO.:19, SEQ ID NO.:84,~~ SEQ ID NO.:96, SEQ ID NO.:98; SEQ ID NO.:104 and SEQ ID NO.:108;
(d) a light chain CDR1 ~~selected from the group~~ of SEQ ID NO.:4~~, SEQ ID NO.:20, SEQ ID NO.:27 and SEQ ID NO.:86~~;
(e) a light chain CDR2 ~~selected from the group~~ of SEQ ID NO.:5~~, SEQ ID NO.:21 and SEQ ID NO.:28~~; and
(f) a light chain CDR3 selected from the group of SEQ ID NO.:6, ~~SEQ ID NO.:22, SEQ ID NO.:87,~~ SEQ ID NO.:99, SEQ ID NO.:105, SEQ ID NO.:109 and SEQ ID NO.:97;
and at least one antigen binding site specific for FAP, comprising
(a) a heavy chain CDR1 selected from the group of SEQ ID NO.:9 and SEQ ID NO.:33;
(b) a heavy chain CDR2 selected from the group of SEQ ID NO.:10 and SEQ ID NO.:34;
(c) a heavy chain CDR3 selected from the group of SEQ ID NO.:11 and SEQ ID NO.:35;
(d) a light chain CDR1 selected from the group of SEQ ID NO.:12 and SEQ ID NO.:36;
(e) a light chain CDR2 selected from the group of SEQ ID NO.:13 and SEQ ID NO.:37;
(f) a light chain CDR3 selected from the group of SEQ ID NO.:14 and SEQ ID NO.:38.

2. The bispecific antibody of claim 1, wherein the antigen binding site specific for DR5 comprises a variable heavy chain and a variable light chain comprising an amino acid sequence selected from the group of: SEQ ID NO.:7 and SEQ ID NO.:8; ~~SEQ ID NO.:23 and SEQ ID NO.:24; SEQ ID NO.:26 and SEQ ID NO.:24; SEQ ID NO.:23 and SEQ ID NO.:29; SEQ ID NO.:23 and SEQ ID NO.:30; SEQ ID NO.:26 and SEQ ID NO.:31; SEQ ID NO.:26 and SEQ ID NO.:32; SEQ ID NO.:26 and SEQ ID NO.:30; SEQ ID NO.:23 and SEQ ID NO.:31; SEQ ID NO.:82 and SEQ ID NO.:85;~~ SEQ ID NO.:100 and SEQ ID NO.:101; SEQ ID NO.:102 and SEQ ID NO.:103; SEQ ID NO.:106 and SEQ ID NO.:107; SEQ ID NO.:94 and SEQ ID NO.:95;

22. The bispecific antibody of any of claims 7 to 21, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

23. The bispecific antibody of claim 22, wherein said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329.

24. The bispecific antibody of claim 23, wherein each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an activating or inhibitory Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G.

25. The bispecific antibody of any of claims 7 to 24, wherein the Fc part of the first heavy chain comprises a first dimerization module and the Fc part of the second heavy chain comprises a second dimerization module allowing a heterodimerization of the two heavy chains of the first antibody.

26. The bispecific antibody of claim 25, wherein the first dimerization module comprises knobs and the second dimerization module comprises holes according to the knobs into holes strategy.

27. An antibody that specifically binds to DR5, comprising

(a) a heavy chain complementarity determining region 1 (CDR1) ~~selected from the group consisting~~ of SEQ ID NO.:1~~, SEQ ID NO.:17 and SEQ ID NO.:75~~;

(b) a heavy chain complementarity determining region 2(CDR2) ~~selected from the group~~ of SEQ ID NO.:2~~, SEQ ID NO.:18, SEQ ID NO.:25 and SEQ ID NO.:83~~;

(c) a heavy chain complementarity determining region 3 (CDR3) selected from the group of SEQ ID NO.:3, ~~SEQ ID NO.:19, SEQ ID NO.:84,~~ SEQ ID NO.:96, SEQ ID NO.:98, SEQ ID NO.:104 and SEQ ID NO.:108;

(d) a light chain CDR1 ~~selected from the group~~ of SEQ ID NO.:4~~, SEQ ID NO.:20, SEQ ID NO.:27 and SEQ ID NO.:86~~;

(e) a light chain CDR2 ~~selected from the group~~ of SEQ ID NO.:5~~, SEQ ID NO.:21 and SEQ ID NO.:28~~; and

(f) a light chain CDR3 selected from the group of SEQ ID NO.:6, ~~SEQ ID NO.:22, SEQ ID NO.:87,~~ SEQ ID NO.:99, SEQ ID NO.:105, SEQ ID NO.:109 and SEQ ID NO.:97.~~;~~

28. The antibody of claim 27, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:1;

(b) a heavy chain CDR2 of SEQ ID NO.:2;

(c) a heavy chain CDR3 of SEQ ID NO.:3;

(d) a light chain CDR1 of SEQ ID NO.:4;

(e) a light chain CDR2 of SEQ ID NO.:5;

(f) a light chain CDR3 of SEQ ID NO.:6.

29. The antibody of claim 27, comprising a variable heavy chain and a variable light chain comprising an amino acid sequence selected from the group of: SEQ ID NO.:7 and SEQ ID NO.:8; ~~SEQ ID NO.:23 and SEQ ID NO.:24; SEQ ID NO.:26 and SEQ ID NO.:24; SEQ ID NO.:23 and SEQ ID NO.:29; SEQ ID NO.:23 and SEQ ID NO.:30; SEQ ID NO.:26 and SEQ ID NO.:31; SEQ ID NO.:26 and SEQ ID NO.:32; SEQ ID NO.:26 and SEQ ID NO.:30; SEQ ID NO.:23 and SEQ ID NO.:31; SEQ ID NO.:82 and SEQ ID NO.:85;~~ SEQ ID NO.:100 and SEQ ID NO.:101; SEQ ID NO.:102 and SEQ ID NO.:103; SEQ ID NO.:106 and SEQ ID NO.:107; SEQ ID NO.:94 and SEQ ID NO.:95.

30. The antibody of any of claims 27 to 29, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:7 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:8.

31. A pharmaceutical composition comprising a bispecific antibody of claims 1 to 26 or the antibody of claims 27 to 30.

32. The bispecific antibody of claims 1 to 26 or the antibody of claims 27 to 30 for the treatment of cancer.

33. The bispecific antibody of claims 1 to 26 or the antibody of claims 27 to 30 for use as a medicament.

34. Use of the bispecific antibody of claims 1 to 26 or the antibody of claims 27 to 30 in the manufacture of a medicament.

35. The use of claim 34, wherein the medicament is for treatment of cancer.

36. The use of claim 34, wherein the medicament is for treatment of pancreatic cancer or colorectal carcinoma.

37. A nucleic acid sequence comprising a sequence encoding a heavy chain ~~of a bispecific antibody of claims 1 to 26 or the heavy chain~~ of an antibody of claims 27 to 30.

38. A nucleic acid sequence comprising a sequence encoding a light chain ~~of a bispecific antibody of claims 1 to 26 or the light chain~~ of an antibody of claims 27 to 30.

39. An expression vector comprising a nucleic acid sequence of claim 37 and/or claim 38.

40. A prokaryotic or eukaryotic host cell comprising a vector according to claim 39.

41. A method of producing an antibody comprising culturing the host cell of claim 40 so that the antibody is produced.