caspase-8 and -10 are then released in the cytosol, where they activate downstream effector caspases such as caspase-3. Activation of effector caspases culminates with apoptotic cell death.

FAPα is a type-II transmembrane (homodimer) serine protease, that is overexpressed in pathological conditions including fibrosis, arthritis, and cancer. FAPα is primarily expressed by activated stromal fibroblasts such as CAFs. FAPα can also be shed from the cell membrane forming soluble FAPα. FAPα is a member of the prolyl peptidase family and shares 70% amino acid sequence homology with the well-described dipeptidyl peptidase 4 (DPP4). Both peptidases contain dipeptidyl peptidase enzymatic activity, whilst endopeptidase activity is FAPα-specific and targets substrates including denatured collagen and α-2 anti-plasmin. While for most cancers elevated FAPα expression is associated with a worse outcome, the underlying biological mechanisms remain poorly understood.

Several first-generation DR agonist antibodies have been tested in the clinic showing limited antitumor efficacy, likely due to their inability to induce efficient receptor clustering which is essential to trigger apoptosis (Dubuisson and Micheau, Antibodies (Basel) 6(4), 2017). One such example is mapatumumab (HGS-ETR1), a DR4-specific agonist monoclonal antibody which showed limited clinical activity as explored in multiple phase 1/2 trials (Snajdauf et al., Front Mol Biosci 8: 628332, 2021). Next-generation agents, such as the TRAIL-R agonist Fc-fusion protein Eftozanermin alfa (ABBV-621), showed encouraging clinical activity but also induced side effects such as liver toxicity (Papadopoulos et al, Cancer Chemother Pharmacol 75(5):887-895, 2015; LoRusso et al., Invest New Drugs 40(4): 762-772, 2022; Di Cristofano et al., Biochem Soc Trans 51(1): 57-70, 2023). The dual targeting of DR and FAPα has been explored with RG7386, an optimized tetravalent bispecific antibody targeting FAPα and DR5 (US9926379B2). However, clinical development for solid malignancies was stopped in 2018. Thus, there is a clear unmet need for development of novel therapeutics with improved safety and efficacy.

Summary

It is an object of the present disclosure to provide multispecific antibodies with improved safety and efficacy.

The multispecific antibodies according to the present disclosure comprise a DR4 binding region and a FAPα binding region. Thus, the proposed mechanism of action for the multispecific antibodies according to the present invention is a conditional transactivation of DR4 as a result of simultaneous binding to DR4-expressing tumor cells

and FAPα on CAFs in the tumor microenvironment (TME). Furthermore, it has been found that some DR4 binding antibodies bind well to DR4-expressing cells and some FAPα binding antibodies bind well to FAPα-expressing cells in a bivalent (monoclonal) format but exhibit reduced binding in monovalent format. Thus, another object of the present disclosure relates to monoclonal antibodies capable of strong binding in either monovalent or bivalent format. This may form the basis for generation of e.g. bispecific antibodies showing strong monovalent binding to DR4 and FAPα.

As demonstrated in the present disclosure e.g. by examples 10-13 and 15, the trans-binding mechanism of the multispecific antibody is advantageous as the killing of the DR4-expressing tumor cell would be dependent on simultaneous binding of the multispecific antibody to a FAPα-expressing cell, such as CAFs. Thus, FAPα-dependent DR4 transactivation results in improved tumor-specific targeting due to the high expression of FAPα on CAFs in the TME. In addition, the multispecific antibody is showing an extended therapeutic window due to trans-binding dependent DR4 activation as opposed to cis-binding as DR4 is not expressed on fibroblasts, (e.g. example 9). As fibroblasts do not express DR4 they are not sensitive to induction of cell death through FAP-dependent DR4 agonism (e.g. example 9 and 11). In one example, the present disclosure relates to a multispecific antibody comprising at least (i) a FAPα binding region capable of binding to FAPα comprising a first heavy chain variable region and a first light chain variable region; and (ii) a DR4 binding region capable of binding to DR4 comprising a second heavy chain variable region and a second light chain variable region.

Accordingly, in one aspect, there is provided a multispecific antibody comprising at least

(i) a FAPα binding region comprising a first heavy chain variable region and a first light chain variable region; and

(ii) a DR4 binding region comprising a second heavy chain variable region and a second light chain variable region.

In a further aspect, the present disclosure relates to a nucleic acid construct, or a combination of nucleic acid constructs, encoding an antibody as defined herein.

In another aspect, there is provided an expression vector, or a combination of expression vectors, comprising the nucleic acid construct(s) as defined herein.

In another aspect, the present disclosure relates to a composition comprising a nucleic acid construct or a combination of nucleic acid constructs as defined herein.

In yet another aspect, the present disclosure relates to a delivery vehicle comprising the nucleic acid construct(s) as described herein.

In still another aspect, the present disclosure relates to a recombinant host cell capable of producing the antibody as described herein, wherein the host cell comprises one or more nucleic acid constructs encoding the antibody as described herein.

In an even further aspect, the present disclosure relates to a pharmaceutical composition comprising a multispecific antibody as defined herein and a pharmaceutically-acceptable carrier.

In another aspect, there is provided the multispecific antibody as defined herein, the nucleic acid construct(s) as defined herein, the delivery vehicle as defined herein or the pharmaceutical composition as defined herein for use as a medicament.

In a still further example, the present disclosure relates to the multispecific antibody as described herein, the nucleic acid construct(s) as described herein, the delivery vehicle as described herein or the pharmaceutical composition as described herein for use in the treatment of cancer.

In a further aspect, there is provided a use of the multispecific antibody as defined herein, the nucleic acid construct(s) as defined herein, the delivery vehicle as defined herein or the pharmaceutical composition as defined herein in the manufacture of a medicament for treating cancer.

In an even further aspect, the present disclosure relates to methods for producing multispecific antibodies according to the disclosure.

Thus, in one aspect, there is provided a method for producing an antibody as defined herein, comprising

(a) culturing the recombinant host cell as defined herein under conditions wherein the antibody is produced, and

(b) isolating the produced antibody from the culture.

In another aspect, there is provided a method for producing a multispecific antibody, comprising

(a) providing

(i) a first antibody as described herein and a second antibody comprising

a DR4 binding region comprising a heavy chain variable region (VH) that comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and a light chain variable region (VL) that comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively;

wherein the antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively;

(ii) a second antibody as described herein and a first antibody comprising

a FAPα binding region comprising a heavy chain variable region (VH) that comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and a light chain variable region (VL) that comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively;

wherein the antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; or

(iii) a first antibody as described herein and a second antibody as described in any one herein;

wherein the sequences of the first and second CH3 regions of the first and second antibodies are different and are such that the heterodimeric interaction between the first and second CH3 regions is stronger than each of the homodimeric interactions of the first and second CH3 regions; wherein preferably the amino acid in the position corresponding to F405 is L in the first CH3 region and the amino acid in the position corresponding to K409 is R in the second CH3 region, or vice versa;

(b) incubating the first antibody together with the second antibody under reducing conditions sufficient to allow the cysteines in the hinge regions to undergo disulfide-bond isomerization; and

(c) obtaining the multispecific antibody comprising the first immunoglobulin heavy chain and the first immunoglobulin light chain of the first antibody and the second immunoglobulin heavy chain and the second immunoglobulin light chain of the second antibody.

In a further aspect, there is provided a multispecific antibody obtained by a method as described herein.

In yet another aspect, there is provided a kit-of-parts, such as a kit for use as a companion diagnostic/for identifying within a population of patients those patients which have a propensity to respond to treatment with an antibody as defined herein, comprising an antibody as defined herein; and instructions for use of said kit.

In still another aspect, there is provided a diagnostic composition comprising an antibody as defined herein, optionally further comprising a dilution buffer.

Finally, the disclosure also provides monospecific antibodies targeting DR4 and FAPα, respectively.

Thus, in one aspect, there is provided an anti-FAPα antibody comprising at least one FAPα binding region, wherein the FAPα binding region comprises a heavy chain variable region (VH) comprising the three complementarity determining regions, CDR1,

CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region (VL) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 14.

In another aspect, there is provided an anti-DR4 antibody comprising at least one DR4 binding region, wherein the DR4 binding region comprises a heavy chain variable region (VH) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 15, and a light chain variable region (VL) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 16.

These and other aspects and embodiments are described in more detail in the following sections.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Binding to human lung fibroblasts and CAFs. (A-B) Binding of BisG1-FAPα-FERL/DR4-FERR, BisG1-FAPα-FERL/b12-FERR, BisG1-b12-FERL/b12-FERR and IgG1-FAPα-FERL to human lung fibroblasts (A) and CAFs (B) was assessed via flow cytometry.Data shown are geomean fluorescence intensity (gMFI) values for one representative experiment out of three. The concentration (µg/mL) at which 50% of the maximal effect was observed (EC_{50}) was derived from the fitted curves. For CAFs (B), IgG1-FAPα-FERL antibody was included with only the top four concentrations. (C) Binding of two FAPα targeting antibody clones, FAPα-FEAL and FAP5 (antibodies BisG1-FAPα-FEAL/b12-FEAR, BisG1-FAP5-FEAL/b12-FEAR, IgG1-FAPα-FEAL and IgG1-FAP5-FEAL) and IgG1-b12-FEAR to human lung fibroblasts was assessed via flow cytometry. Data shown are gMFI values as determined by flow cytometry, for one representative experiment out of two.

Figure 2: Binding of DR4-specific antibodies to cell surface expressed DR4. (A-F) Binding of BisG1-FAPα-FERL/DR4-FERR, BisG1-b12-FERL/DR4-FERR, IgG1-DR4-FERR and IgG1-b12 was assessed on six different cell lines: Colorectal adenocarcinoma cell lines DLD-1 and HCT-15, lung carcinoma A549, colorectal carcinoma HCT-116, breast adenocarcinoma MDA-MB-231 and pancreatic ductal adenocarcinoma PANC-1. Data shown are gMFI values as determined by flow cytometry, for one representative experiment out of three. (G) Binding of BisG1-b12-FEAL/DR4-T1014A04-FEAR, BisG1-

b12-FEAL/DR4-chCTB007-FEAR, IgG1-DR4-chCTB007-FEAR, IgG1-DR4-T1014A04-FEAR and IgG1-b12-FEAL to DR4 was assessed on the multiple myeloma cell line OPM-2. Data shown are gMFI values as determined by flow cytometry, for one experiment. (H) Binding of IgG1-DR4-FERR, BisG1-b12-FERL/DR4-FERR, BisG1-FAPα-FERL/DR4-FERR, IgG1-

CLAIMS

1. A multispecific antibody comprising at least

(i) a FAPα binding region comprising a first heavy chain variable region and a first light chain variable region; and

(ii) a DR4 binding region comprising a second heavy chain variable region and a second light chain variable region.

2. The multispecific antibody according to any one of the preceding claims, wherein the FAPα binding region comprises a heavy chain variable region (VH) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region (VL) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 14.

3. The multispecific antibody according to any one of the preceding claims, wherein the FAPα binding region comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and a light chain variable region (VL) comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively.

4. The multispecific antibody according to any of the preceding claims, wherein

(a) the VH sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 13;

(b) said VL sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 14;

(c) VH sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 13 and said VL sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 14;

(d) the framework regions of the VH sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 13;

(e) the framework regions of the VL sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 14;

(f) the framework regions of the VH sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 13 and said framework regions of the VL sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 14

(g) said VH sequence of the FAPα binding region deviate from SEQ ID NO.: 13 by at the most 10 substitutions, such as at the most 9 substitutions, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution; and/or.

(h) VL sequence of the FAPα binding region deviate from SEQ ID NO.: 14 by at the most 10 substitutions, such as at the most 9 substitution, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution.

5. The multispecific antibody according to any of the preceding claims, wherein

(a) the VH sequence of the FAPα binding region comprises, consists essentially of or consists of a VH sequence as set forth in SEQ ID NO.: 13;

(b) the VL sequence of the FAPα binding region comprises, consists essentially of or consists of a VL sequence as set forth in SEQ ID NO.: 14; or

(c) the VH and VL sequences of the FAPα binding region comprise, consist essentially of or consist of a VH sequence as set forth in SEQ ID NO.: 13 and a VL sequence as set forth in SEQ ID NO.: 14.

6. The multispecific antibody according to any of the preceding claims, wherein the DR4 binding region comprises a heavy chain variable region (VH) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 15, and a light chain variable region (VL) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 16.

7. The multispecific antibody according to any one of the preceding claims, wherein the DR4 binding region comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and a light chain variable region (VL) comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively.

8. The multispecific antibody according to any of the preceding claims, wherein

(a) the VH sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 15;

(b) said VL sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 16; or

(c) the VH sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 15 and said VL sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 16;

(d) the framework regions of the VH sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 15;

(e) the framework regions of the VL sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 16;

(f) the framework regions of the VH sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 15 and said framework regions of the VL sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the framework regions of the amino acid sequence as set forth in SEQ ID NO.: 16;

(g) said VH sequence of the DR4 binding region deviate from SEQ ID NO.: 15 by at the most 10 substitutions, such as at the most 9 substitutions, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution; and/or

(h) said VL sequence of the DR4 binding region deviate from SEQ ID NO.: 16 by at the most 10 substitutions, such as at the most 9 substitution, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution.

9. The multispecific antibody according to any of the preceding claims, wherein

(a) the VH sequence of the DR4 binding region comprises, consists essentially of or consists of a VH sequence as set forth in SEQ ID NO.: 15;

(b) the VL sequence of the DR4 binding region comprises, consists essentially of or consists of a VL sequence as set forth in SEQ ID NO.: 16; or

(c) the VH and VL sequences of the DR4 binding region comprise, consist essentially of or consist of a VH sequence as set forth in SEQ ID NO.: 15 and a VL sequence as set forth in SEQ ID NO.: 16.

10. The multispecific antibody according to any one of the preceding claims, wherein said antibody is a bispecific antibody.

11. The multispecfic antibody according to any of the preceding claims, wherein the K_D of FAPα binding to the FAPα binding region is 100 pM or less, and the K_D of DR4 binding to the DR4 binding region is 1 nM or less.

12. The multispecific antibody according to any of the preceding claims, wherein said antibody comprises

(i) a FAPα binding region comprising a first heavy chain variable region and a first light chain variable region, wherein the heavy chain variable region (VH) comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and the light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively; and

(ii) a DR4 binding region comprising a second heavy chain variable region and a second light chain variable region, wherein the heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and the light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively.

13. The multispecific antibody according to any of the preceding claims, wherein said antibody comprises

(i) a FAPα binding region comprising, consisting essentially of or consisting of a VH sequence as set forth in SEQ ID NO.: 13 and a VL sequence as set forth in SEQ ID NO.: 14, and

(ii) a DR4 binding region comprising, consisting essentially of or consisting of a VH sequence as set forth in SEQ ID NO.: 15 and a VL sequence as set forth in SEQ ID NO.: 16.

14. The multispecific antibody according to any one of the preceding claims,

(i) wherein the antibody comprises a first heavy chain and a second heavy chain, said first heavy chain comprising said VH region of the FAPα binding region and said second heavy chain comprising said VH region of the DR4 binding region

(ii) wherein each of said first heavy chain and said second heavy chain comprises at least a hinge region, a CH2 and a CH3 region, and

(iii) wherein in said first heavy chain at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 (in a human IgG1 heavy chain according to Eu numbering) has been substituted, and in said second heavy chain at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 (in a human IgG1 heavy chain according to Eu numbering) has been substituted.

15. The multispecific antibody according to any of the preceding claims, wherein

(i) the amino acid in the position corresponding to F405 (in a human IgG1 heavy chain according to Eu numbering) is L in said first heavy chain, and the amino acid in the position corresponding to K409 (in a human IgG1 heavy chain according to Eu numbering) is R in said second heavy chain, or

(ii) the amino acid in the position corresponding to K409 (in a human IgG1 heavy chain according to Eu numbering) is R in said first heavy chain, and the amino acid in the position corresponding to F405 (in a human IgG1 heavy chain according to Eu numbering) is L in said second heavy chain.

16. The multispecific antibody according to any one of the preceding claims, wherein said antibody comprises a first heavy chain and a second heavy chain, wherein in at least one of said first heavy chain and said second heavy chain one or more amino acids in the positions corresponding to positions L234, L235, G236, D265, N297, and P331 in a human IgG1 heavy chain according to Eu numbering, are not L, L, G, D, N, and P, respectively.

17. The multispecific antibody according to any of the preceding claims, wherein

(a) the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to Eu numbering are F and E, respectively, in said first and/or second heavy chains;

(b) the antibody is a bispecific antibody comprising a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F and E, respectively, and wherein

(i) the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is R, or

(ii) the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is L;

(c) the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to Eu numbering are F, E, and A, respectively, in said first and/or second heavy chains;

(d) the antibody is a bispecific antibody comprising a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and A, respectively, and wherein

(i) the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is R, or

(ii) the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is L;

(e) the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering are F, E, and R, respectively, in said first and/or second heavy chains;

~~(e)~~(f) the antibody is a bispecific antibody comprising a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively, and wherein

(i) the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is R, or

(ii) the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is L;

~~(f)~~(g) one of the first and second heavy chains comprises substitutions of the amino acids corresponding to the amino acids at positions L234, L235 and G236 to F, E and R, respectively, and the other heavy chain comprises substitutions of

the amino acids corresponding to the amino acids at positions L234, L235 and D265 to F, E and A, respectively, wherein the amino acid positions are as defined by Eu numbering; or

~~(f)~~(h) the antibody is a bispecific antibody comprising a first heavy chain and a second heavy chain and wherein one heavy chain comprises substitutions of the amino acids corresponding to the amino acids at positions L234, L235 and G236 to F, E and R, respectively, and the other heavy chain comprises substitutions of the amino acids corresponding to the amino acids at positions L234, L235 and D265 to F, E and A, respectively, and wherein

(i) the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is R, or

(ii) the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R, and the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is L.

18. The multispecific antibody according to any one of the preceding claims, wherein

(a) said antibody is an IgG1 antibody;

(b) the antibody is a full-length antibody; and/or

~~(b)~~(c) the antibody is a full-length IgG1 antibody.

19. The multispecific antibody according to any one of the preceding claims, wherein said antibody comprises a heavy chain and a kappa (κ) light chain which comprise the FAPα binding region and a heavy chain and a lambda (λ) light chain which comprise the DR4 binding region.

20. The multispecific antibody according to any one of the preceding claims, wherein said antibody, optionally is a bispecific antibody, and comprises

(i) a first heavy chain and a first light chain comprising a FAPα binding region, wherein the FAPα binding region comprises a first heavy chain variable region and a first light chain variable region, wherein the first heavy chain variable region (VH) comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.:

1, 2 and 3, respectively, and the first light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively;

(ii) a second heavy chain and a second light chain comprising a DR4 binding region, wherein the DR4 binding region comprises a second heavy chain variable region and a second light chain variable region, wherein the second heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and the second light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively;

(iii) wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; and

(iv) wherein the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L, and the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the second heavy chain is R.

21. The multispecific antibody according to any of the preceding claims, wherein said antibody, optionally being a bispecific antibody, comprises

(i) a FAPα heavy chain sequence as set forth in SEQ ID NO.: 17 and a FAPα light chain sequence as set forth in SEQ ID NO.: 18, and

(ii) a DR4 heavy chain sequence as set forth in SEQ ID NO.: 19 and a DR4 light chain sequence as set forth in SEQ ID NO.: 20.

22. The multispecific antibody according to any of the preceding claims, wherein said multispecific antibody is a bispecific, bivalent antibody having monovalent binding to FAPα and monovalent binding to DR4.

23. A nucleic acid construct, or a combination of nucleic acid constructs, encoding an antibody as defined in any of the preceding claims.

24. An expression vector, or a combination of expression vectors, comprising the nucleic acid construct(s) according to claim 23.

25. A composition comprising a nucleic acid construct or a combination of nucleic acid constructs as defined in claim 23.

26. A delivery vehicle comprising the nucleic acid construct(s) according to claim 23.

27. A recombinant host cell capable of producing the antibody according to any one of the claims 1-22, wherein the host cell comprises one or more nucleic acid constructs encoding the antibody as defined in any of the claims 1-22, optionally wherein the recombinant host cell is a CHO cell.

28. A pharmaceutical composition comprising a multispecific antibody as defined in any one of the claims 1-22 and a pharmaceutically-acceptable carrier.

29. The multispecific antibody as defined in any of claims 1-22, the nucleic acid construct(s) according to claim 23, the delivery vehicle according to claims 26 or the pharmaceutical composition according to claim 28 for use as a medicament.

30. Use of the multispecific antibody as defined in any of claims 1-22, the nucleic acid construct(s) according to 23, the delivery vehicle according to claim 26 or the pharmaceutical composition according to claim 28 in the manufacture of a medicament for treating cancer.

31. Use of the multispecific antibody as defined in any of claims 1-22, the nucleic acid construct(s) according to claim 23, the delivery vehicle according to claim 26 or the pharmaceutical composition according to claim 28 in the manufacture of a medicament for treating a primary tumor and/or for preventing and treating metastases.

32. The use according to any one of the claims 30-31, wherein

(a) the cancer is solid cancer, such as a malignant solid tumor e.g., an advanced and/or a metastatic solid tumor; and/or a carcinoma;

(b) the cancer is selected from the group consisting of colorectal cancer [CRC]; breast cancer, such as triple negative breast cancer [TNBC]; pancreatic cancer, such as pancreatic ductal adenocarcinoma [PDAC]; esophagogastric cancer, such as gastric cancer and esophageal cancer; Head and neck squamous cell carcinoma [HNSCC]; cervical cancer; and lung cancer, such as non-small cell lung cancer [NSCLC]; and/or

(c) the cancer is selected from the group consisting of pancreatic cancer, gastric cancer and CRC.

33. A method for producing an antibody as defined in any one of the claims 1-22, comprising

(a) culturing the recombinant host cell of claim 27 under conditions wherein the antibody is produced, and

(b) isolating the produced antibody from the culture.

34. A kit-of-parts, such as a kit for use as a companion diagnostic/for identifying within a population of patients those patients which have a propensity to respond to treatment with an antibody as defined in any one of the claims 1-22, comprising an antibody as defined in any one of claims 1-22; and instructions for use of said kit.

35. A diagnostic composition comprising an antibody as defined in any one of the claims 1-22, optionally further comprising a dilution buffer.

36. An anti-FAPα antibody comprising at least one FAPα binding region, wherein the FAPα binding region comprises a heavy chain variable region (VH) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 13, and a light chain variable region (VL) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 14.

37. The anti-FAPα antibody according to claim 36, wherein the anti-FAPα antibody comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and a light chain variable region (VL) comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively.

38. The anti-FAPα antibody according to any one of the claims 36-37, wherein

(a) the VH sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 13;

(b) said VL sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 14;

(c) the VH sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 13 and said VL sequence of the FAPα binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 14;

(d) said VH sequence of the FAPα binding region deviate from SEQ ID NO.: 13 by at the most 10 substitutions, such as at the most 9 substitutions, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at

the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution; and/or

(e) said VL sequence of the FAPα binding region deviate from SEQ ID NO.: 14 by at the most 10 substitutions, such as at the most 9 substitutions, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution.

39. The anti-FAPα antibody according to any one of the claims 36-38,

(i) wherein the anti-FAPα antibody comprises a first heavy chain and a second heavy chain,

(ii) wherein each of said first heavy chain and said second heavy chain comprises at least a hinge region, a CH2 and a CH3 region,

(iii) wherein in said first heavy chain at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 (in a human IgG1 heavy chain according to Eu numbering) has been substituted, and in said second heavy chain at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 (in a human IgG1 heavy chain according to Eu numbering) has been substituted; such as

wherein

(i) the amino acid in the position corresponding to F405 (in a human IgG1 heavy chain according to Eu numbering) is L, or

(ii) the amino acid in the position corresponding to K409 (in a human IgG1 heavy chain according to Eu numbering) is R.

40. The anti-FAPα antibody according to any one of the claims 36-39, wherein

(a) the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to Eu numbering are F and E, respectively, in said first heavy chain and second heavy chain;

(b) the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to Eu numbering are F, E, and A, respectively, in said first heavy chain and said second heavy chain; or

(c) the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering are F, E, and R, respectively, in said first heavy chain and said second heavy chain.

41. The anti-FAPα antibody according to any one of the claims 36-39, wherein the anti-FAPα antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively, and wherein

(i) the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering is L, or

(ii) the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R.

42. The anti-FAPα antibody according to any one of the claims 36-41, wherein

(a) the antibody comprises

(i) a FAPα binding region, wherein the heavy chain variable region (VH) comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and the light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively;

(ii) wherein the anti-FAPα antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; and

(iii) wherein the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L, or wherein the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R; or

(b) the antibody comprises

(i) a FAPα binding region, wherein the heavy chain variable region (VH) comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and the light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively;

(ii) wherein the anti-FAPα antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; and

(iii) wherein the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is L.

43. The anti-FAPα antibody according to any one of the claims 36-42, wherein said antibody comprises a heavy chain sequence as set forth in SEQ ID NO.: 17 and a light chain sequence as set forth in SEQ ID NO.: 18.

44. An anti-DR4 antibody comprising at least one DR4 binding region, wherein the DR4 binding region comprises a heavy chain variable region (VH) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 15, and a light chain variable region (VL) comprising the three complementarity determining regions, CDR1, CDR2, and CDR3, present within the amino acid sequence set forth in SEQ ID NO: 16.

45. The anti-DR4 antibody according to claim 44, wherein the DR4 binding region comprises a heavy chain variable region (VH) comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and a light chain variable region (VL) comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively.

46. The anti-DR4 antibody according to any one of the claims 44-45, wherein

(a) the VH sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 15;

(b) said VL sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 16;

(c) the VH sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 15 and said VL sequence of the DR4 binding region has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity to the amino acid sequence as set forth in SEQ ID NO.: 16;

(d) said VH sequence of the DR4 binding region deviate from SEQ ID NO.: 15 by at the most 10 substitutions, such as at the most 9 substitutions, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution; and/or

(e) said VL sequence of the DR4 binding region deviate from SEQ ID NO.: 16 by at the most 10 substitutions, such as at the most 9 substitutions, like at the most 8 substitutions, such as at the most 7 substitutions, like at the most 6 substitutions, such as at the most 5 substitutions, like at the most 4 substitutions, such as at the most 3 substitutions, like at the most 2 substitutions, such as at the most 1 substitution.

47. The anti-DR4 antibody according to any one of the claims 44-46,

(i) wherein the anti-DR4 antibody comprises a first heavy chain and a second heavy chain,

(ii) wherein each of said first heavy chain and said second heavy chain comprises at least a hinge region, a CH2 and a CH3 region,

(iii) wherein in said first heavy chain at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 (in a human IgG1 heavy chain according to Eu numbering) has been substituted, and in said second heavy chain at least one of the amino acids in a position corresponding to a position selected from the group consisting of T366, L368, K370, D399, F405, Y407, and K409 (in a human IgG1 heavy chain according to Eu numbering) has been substituted, such as

wherein

(i) the amino acid in the position corresponding to F405 (in a human IgG1 heavy chain according to Eu numbering) is L, or

(ii) the amino acid in the position corresponding to K409 (in a human IgG1 heavy chain according to Eu numbering) is R.

48. The anti-DR4 antibody according to any one of the claims 44-47, wherein

(a) the positions corresponding to positions L234 and L235 in a human IgG1 heavy chain according to Eu numbering are F and E, respectively, in said first heavy chain and said second heavy chain;

(b) the positions corresponding to positions L234, L235, and D265 in a human IgG1 heavy chain according to Eu numbering are F, E, and A, respectively, in said first heavy chain and said second heavy chain; or

(c) the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering are F, E, and R, respectively, in said first heavy chain and said second heavy chain.

49. The anti-DR4 antibody according to any one of the claims 44-48, wherein the anti-DR4 antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively, and wherein

(i) the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering is L, or

(ii) the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering of the first heavy chain is R.

50. The anti-DR4 antibody according to any one of the claims 44-49, wherein

(a) the antibody comprises

(i) a DR4 binding region, wherein the heavy chain variable region (VH) comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and the light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively;

(ii) wherein the anti-DR4 antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; and

(iii) wherein the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering is R in the first and second heavy chains, or wherein the position corresponding to F405 in a human IgG1 heavy chain according to Eu numbering is L in the first and second heavy chains; or

(b) the antibody comprises

(i) a DR4 binding region, wherein the heavy chain variable region (VH) comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and the light chain variable region (VL) comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively;

(ii) wherein the anti-DR4 antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; and

(iii) wherein the position corresponding to K409 in a human IgG1 heavy chain according to Eu numbering is R in the first and second heavy chains.

51. The anti-DR4 antibody according to any one of the claims 44-50, wherein said antibody comprises a heavy chain sequence as set forth in SEQ ID NO.: 19 and a light chain sequence as set forth in SEQ ID NO.: 20.

52. A method for producing a multispecific antibody, comprising

(a) providing

(i) a first antibody as described in any one of the claims 36-43 and a second antibody comprising

a DR4 binding region comprising a heavy chain variable region (VH) that comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 7, 8 and 9, respectively, and a light chain variable region (VL) that comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 10, 11 and 12, respectively;

wherein the antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively;

(ii) a second antibody as described in any one of the claims 44-51 and a first antibody comprising

a FAPα binding region comprising a heavy chain variable region (VH) that comprises the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs.: 1, 2 and 3, respectively, and a light chain variable region (VL) that comprises the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs.: 4, 5 and 6, respectively;

wherein the antibody comprises a first heavy chain and a second heavy chain and wherein the positions corresponding to positions L234, L235, and G236 in a human IgG1 heavy chain according to Eu numbering of both the first heavy chain and the second heavy chain are F, E, and R, respectively; or

(iii) a first antibody as described in any one of the claims 36-43 and a second antibody as described in any one of the claims 44-51;

wherein the sequences of the first and second CH3 regions of the first and second antibodies are different and are such that the heterodimeric interaction between the first and second CH3 regions is stronger than each of the homodimeric interactions of the first and second CH3 regions; wherein preferably the amino acid in the position corresponding to F405 is L in the first CH3 region and the amino acid in the position corresponding to K409 is R in the second CH3 region, or vice versa;

(b) incubating the first antibody together with the second antibody under reducing conditions sufficient to allow the cysteines in the hinge regions to undergo disulfide-bond isomerization; and

(c) obtaining the multispecific antibody comprising the first immunoglobulin heavy chain and the first immunoglobulin light chain of the first antibody and the second immunoglobulin heavy chain and the second immunoglobulin light chain of the second antibody.

53. A multispecific antibody obtained by a method as described in claim 52.