BISPECIFIC ANTIBODIES SPECIFIC FOR FAP AND DR5, ANTIBODIES SPECIFIC FOR DR5 AND METHODS OF USE

FIELD OF THE INVENTION

The present invention relates to bispecific antibodies comprising a first antigen binding site specific for Death Receptor 5 (DR5) and a second antigen binding site specific for Fibroblast Activation Protein (FAP), antibodies specific for DR5, methods for their production, pharmaceutical compositions containing said antibodies, and uses thereof.

BACKGROUND

Monoclonal antibodies are powerful therapeutic agents in the treatment of cancer since they selectively target antigens which are differentially expressed on cancer cells. Targeting of the TRAIL (TNF related apoptosis inducing ligand) death receptors on cancer cells with agonistic monoclonal antibodies represents a new generation of monoclonal antibody therapy, as they are able to directly induce apoptosis of targeted cells.

Upon binding of TRAIL, death receptors of the TNFR-SF family such as DR4 and DR5 become trimerized. The trimerization induces the extrinsic apoptotic pathway and a complex cascade of events including Caspase activation, which finally result in the killing of the target cells. Apoptosis induction is further enhanced if hyperclustering of DR5 (i.e. the clustering of multiple trimers) takes place. Although death receptors are widely expressed on a variety of cell types, induction of apoptosis via the extrinsic pathway is restricted to tumor cells. Since agonistic DR4 or DR5 binding antibodies are able to cross-link death receptors and hence induce apoptosis, these receptors are interesting targets in cancer therapy. At least eight death receptor targeting molecules entered clinical development and have been assessed in clinical trials for possible treatment of different indications such as advanced solid tumors like colorectal or lung cancers. In addition there have been attempts to treat other indications such as lymphoma and / or multiple myeloma.

Drozitumab, a fully human DR5 agonistic antibody described in US2007 / 0031414 A1 and WO2006/083971, shows some in vitro apoptotic activity in the absence of cross-linking at high concentrations. However, in vivo data revealed a different mode of action: In FcγR mutant mice (or when antibody variants were used in which FcγR binding was inhibited) Drozitumab was inactive indicating that the in vivo activity of this molecule is mainly dependent on FcγR

mediated cross-linking. This molecule was tested up to clinical phase II, seemed to be save (no MTD up to 20 mg/kg was reached) but did not demonstrate any significant efficacy.

Conatumumab (described in EP1922337A), is another fully human DR5 agonistic antibody. The activity of Conatumumab is strictly dependent on cross-linking via Fc receptors. In contrast to Drozitumab this antibody is non-ligand blocking. Also this molecule only showed very limited efficacy in clinical trials.

LBY-135, a chimeric DR5 antibody, exhibits similar characteristics as Conatumumab with respect to cross-linking dependent activity and non-ligand blocking property and did not demonstrate any significant efficacy in monotherapy. In addition, LBY-135 showed signs of immunogenicity in part of the enrolled patients of a phase I trial.

Dulanermin, a recombinantly produced natural ligand of DR4 and DR5 (TRAIL), only showed limited objective responses in clinical trials. The use of the natural ligand has somehow disadvantageous: TRAIL targets multiple receptors including both the death receptors and decoy receptors and, therefore, selectivity is a concern. In addition, TRAIL has a much shorter blood half-life compared with monoclonal anti- DR antibodies, a factor which affects dose and schedule parameters. The very short blood half-life of TRAIL requires large and frequent doses compared with monoclonal anti-DR antibodies. In addition recombinant TRAIL is very difficult and tedious to produce.

The development of all three DR5 agonistic antibodies and the ligand described above was discontinued.

Two additional fully human antibodies, Mapatumumab (anti DR4) and Lexatumumab (anti DR5) are still in development although also these molecules did not exhibit promising efficacy in monotherapy.

Tigatuzumab is a humanized DR5 agonistic antibody which is described as being active in vitro (already at low concentrations) in the absence of secondary cross-linking which of course bears the risk of systemic toxicity issues. However, as all the other described agonistic DR5 antibodies, also this molecule has not demonstrated convincing efficacy in Ph I / Ph II studies so far and the maximally tolerated dose MTD only was demonstrated up to 8 mg/kg.

A different approach to induce apoptosis by targeting a death receptor is pursued with the molecule TAS266, a tetrameric DR5 binding nanobody (WO2011098520A1). Due to the tetravalent configuration of DR5 binding moieties, it is thought that DR5 cross-linking is increased compared to standard bivalent antibodies, which may result in increased activity. However, due to their small size, these molecules have the disadvantage of a rather short half-life

(compared to antibodies). In addition there is an increased risk of systemic toxicity since this tetrameric molecule is not targeted to the tumor.

Combining the DR5 antibody Drozitumab with a tumor antigen binding moiety or an antigen present in the stroma surrounding the tumor in a bispecific antibody platform has been described by the inventors of the present application as a new approach to achieve two effects: firstly the DR5 binding antibody can be targeted to the tumor site which could avoid potential systemic toxicity issues (especially when using a DR5 antibody exhibiting cross-linking independent activity). Secondly, this tumor or tumor stroma targeting moiety then also serves as the cross-linking unit to induce DR5 hyperclustering and subsequently tumor site specific apoptosis. The basic concept has been demonstrated using Drozitumab_scFv fusion molecules targeting different tumor types (see WO 2011/039126).

Of particular interest therein were bispecific antibodies binding DR5 and Human Fibroblast Activation Protein (FAP; GenBank Accession Number AAC51668). Human FAP was originally identified in cultured fibroblasts using the monoclonal antibody (mAb) F19 (described in WO 93/05804, ATCC Number HB 8269). Homologues of the protein were found in several species, including mice (Niedermeyer et al., Int J Cancer 71, 383-389 (1997), Niedermeyer et al., Eur J Biochem 254, 650-654 (1998); GenBank Accession Number AAH19190). FAP has a unique tissue distribution: its expression was found to be highly upregulated on reactive stromal fibroblasts of more than 90% of all primary and metastatic epithelial tumors, including lung, colorectal, bladder, ovarian and breast carcinomas, while it is generally absent from normal adult tissues (Rettig et al., Proc Natl Acad Sci USA 85, 3110-3114 (1988); Garin-Chesa et al., Proc Natl Acad Sci USA 87, 7235-7239 (1990)). Subsequent reports showed that FAP is not only expressed in stromal fibroblasts but also in some types of malignant cells of epithelial origin, and that FAP expression directly correlates with the malignant phenotype (Jin et al., Anticancer Res 23, 3195-3198 (2003)). Surprisingly the inventors found that a bispecific antibody targeting FAP in the stroma and DR5 on the tumor cell actually induces apoptosis despite the targets being situated on different cells.

Upon further investigation the inventors of the present application found that the scFv containing bispecific molecules described in WO2011/039126 all have some intrinsic issues with respect to productivity, stability and aggregate formation leading to suboptimal, non-specific activity.

In the present application, novel bispecific antibodies targeting FAP and DR5 are provided. The inventors of the present application developed novel DR5 binding moieties that are only active after crosslinking. Hence induction of tumor cell apoptosis is dependent on DR5 hypercrosslinking via FAP and is independent on Fc/ FcR interactions. Therefore in addition to

bispecific antibodies specific for FAP and DR5, also novel antibodies binding to DR5 are provided therein.

In contrast, the activity of conventional DR5 targeting molecules as described above is dependent on Fc Receptor (FcR) mediated hyperclustering, and is influenced by the immune infiltration and activation status in the tumor (Li and Ravetch, PNAS 2012; Wilson, Cancer Cell 2011; WO2011098520A1). The Fc/FcR interactions can be impaired by physiological human IgG levels. Thus the activity of conventional DR5 targeting molecules is often limited to a few infiltrating cells (Moessner, Blood 2010). By using a bispecific antibody targeting both DR5 and FAP, the percentage of sensitive tumor cells can be significantly increased by hypercrosslinking via FAP and the risk of an intrinsic resistance to DR5 agonists is decreased. The novel DR5 binding moieties are only active after crosslinking with FAP, which could result in an improved safety and toxicology profile compared to the DR5 binders Apomab and Tigatuzumab. The DR5 agonists that have been tested so far were safe in the clinic, however, these clinical programs have been impeded by a low efficacy of the DR5 targeting molecules.

In addition, the preferred novel DR5 binding moieties bind to a different epitope than Drozitumab.

Importantly the novel DR5 binding moieties can be employed in many bispecific DR5-FAP targeting antibody formats, including both novel and established bispecific formats. In contrast, only C-terminal fusions of a FAP binding moiety are possible with Drozitumab-based bispecific DR5-FAP targeting antibody formats, as any N-terminal fusion to Drozitumab results in inactive molecules. The provision of the new DR5 binding moieties thus significantly expands the possibilities of employing the DR5 targeting moiety in various bispecific DR5-FAP targeting antibody formats. This is particularly important as some bispecific antibody formats have superior characteristics in terms of producability and activity.

Provided therein are novel bispecific antibodies comprising novel DR5 binding moieties and a affinity matured FAP binding moiety.

The bispecific antibodies of the present invention are provided in a bispecific antibody format, wherein one or more crossover- Fab fragments are fused to an IgG molecule. Crossover Fab fragments are Fab fragments wherein either the variable regions or the constant regions of the heavy and light chain are exchanged. Bispecific antibody formats comprising crossover Fab fragments have been described, for example, in WO2009080252, WO2009080253, WO2009080251, WO2009080254, WO2010/136172, WO2010/145792 and WO2013/026831.

So far, only DR5-FAP bispecific antibodies in a scFv containing format have been described (WO2011/039126). In addition to the advantageous properties of the novel DR5

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 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 developed novel DR5 binding moieties with superior properties compared to known DR5 binders that are incorporated into novel and advantageous DR5-FAP bispecific antibodies.

The 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 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 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;

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

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5 comprising a variable heavy chain and a variable light chain comprising an amino acid sequence 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;
and at least one antigen binding site specific for FAP comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:15 and SEQ ID NO.:39 ; and a variable light chain comprising an amino acid sequence of SEQ ID NO.:16 and SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5 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; and at least one antigen binding site specific for FAP comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

Preferably said bispecific antibody is human or humanized.

In one embodiment, the bispecific antibody comprises an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP.

In one embodiment, the bispecific antibody comprises an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP, wherein at least one of the Fab fragments is connected to the first or second subunit of the Fc domain via the light chain (VLCL) and at least one Fab

fragment is connected to the first or second subunit of the Fc domain via the heavy chain (VHCH1).

In one embodiment, the bispecific antibody comprises
a) an Fc domain,
b) two Fab fragments comprising an antigen binding site specific for DR5,
wherein said Fab fragments are connected at the C-terminus of the constant light chain (CL) to the first or second subunit of the Fc domain,
c) two Fab fragments comprising the antigen binding site specific for FAP, wherein the two Fab fragments are connected at the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain.

In one embodiment, the bispecific antibody comprises
a) an Fc domain,
b) two Fab fragments comprising an antigen binding site specific for DR5,
wherein said Fab fragments are connected at the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain,
c) two Fab fragments comprising the antigen binding site specific for FAP, wherein the two Fab fragments are connected at the C-terminus of the constant light chain (CL) to the first or second subunit of the Fc domain.

In one embodiment, the bispecific antibody comprises an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.
In one embodiment, the bispecific antibody comprises an Fc domain, two Fab fragments comprising each an antigen binding site specific for DR5, and two Fab fragments comprising each an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

In one embodiment said bispecific antibody is bivalent both for DR5 and FAP.

In one embodiment, the bispecific antibody comprises an Fc domain, two Fab fragments comprising each an antigen binding site specific for DR5, and one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

In one embodiment said bispecific antibody is bivalent for DR5 and monovalent for FAP.

In one embodiment, the bispecific antibody comprises an Fc domain, three Fab fragments comprising each an antigen binding site specific for DR5, and one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

In one embodiment said bispecific antibody is trivalent for DR5 and monovalent for FAP.

In one embodiment, the bispecific antibody comprises an Fc domain, one Fab fragment comprising an antigen binding site specific for DR5, and one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

In one embodiment said bispecific antibody is monovalent for DR5 and monovalent for FAP.

In one embodiment said bispecific antibody comprises an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of the Fab fragment(s) comprising an antigen binding site specific for FAP are exchanged.

In one embodiment at least one of said Fab fragments is connected to the Fc domain via a peptide linker.

In one embodiment said bispecific antibody comprises an Fc domain, which comprises one or more amino acid substitution that reduces binding to Fc receptors and/or effector function. In one embodiment said one or more amino acid substitution is at one or more positions selected from the group of L234, L235, and P329. In one embodiment each subunit of the Fc domain

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 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.

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

In another aspect, the present application relates to a bispecific antibody targeting DR5 and FAP or an antibody that specifically binds to DR5 of the present application 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 for the treatment of cancer.

The present application 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. In another aspect, the present application relates to isolated polynucleotides encoding a heavy chain and a light chain of an antibody that specifically binds to DR5 of the present application. The present application also relates toa 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. In another aspect, the present application relates to an expression vector comprising a nucleic acid sequence of the present application. In another aspect, the present application relates to a prokaryotic or eukaryotic host cell comprising a vector of the present application. In another aspect, the present application relates to a

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

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

measured with different concentrations of an anti FAP antibody is shown over a range of three magnitudes (black, grey and hatched bars). Negative control reactions as secondary antibody and cells only are shown as stippled and white bars, respectively. While the GM05389 cells demonstrate expression of FAP over all tested antibody concentrations that was above background, with the SW872 cells FAP expression only could be detected with the highest antibody concentration used (10 µg / ml), indicating that these cells are not suitable for FAP based binding / apoptosis induction experiments. In addition it is shown that this cell line hardly undergoes Drozitumab mediated apoptosis (B). Drozitumab alone or another, commercially available anti DR5 antibody did not induce relevant DNA fragmentation. Only when Drozitumab is cross-linked with an anti-human Fc antibody a detectable low level apoptosis induction can be observed.

Figure 2: Detection of DR5 expression on two different human tumor cell lines (breast cancer cell line MDA-MB-231 and the renal carcinoma cell line ACHN) via FACS binding with Drozitumab and subsequent detection with a labeled anti Fc antibody. Both cell lines show comparable, low expression levels of human DR5.

Figure 3: DNA fragmentation ELISA assay for detection of apoptosis. ACHN (A) or MDA-MB-231 (B) target cells were either cultivated alone or in presence of an equal number of FAP expressing GM05389 fibroblasts. DR5-FAP bispecific antibodies (scFv fusions) were added at a concentration of 0.1 µg / ml (A) and 0.7 nM , respectively (B) and cells were incubated for 24 h prior to detection of DNA fragmentation. For cross-linking of Drozitumab, 0.1 µg / ml secondary anti Fc antibody was used.

Figure 4: DNA fragmentation ELISA assay for detection of apoptosis. Apoptosis induction on ACHN cells by bispecific DR5-FAP antibodies (Drozitumab with fused scFab) in the presence or absence of FAP expressing fibroblasts (GM05389). Activity of bispecific molecules with different FAP binding moieties are compared to Drozitumab with or without cross-linking by a secondary anti Fc antibody. Antibodies were used in two different concentrations (1.0 µg/ml and 0.1 µg/ml). In these settings bispecific molecules containing the 4G8 scFab fusion (B) showed superior apoptosis induction activity in a DNA fragmentation assay compared to the 3F2 scFab containing molecules (A).

Figure 5: Schematic representation of two different bispecific DR5-FAP molecules in which the anti FAP CrossFab moiety by a standard $(G_4S)_4$ linker is fused to the C-terminus of Drozitumab IgG. In one case (A) the VHCL CrossFab is fused to Drozitumab IgG while in (B) the VLCH1 chain is connected to the IgG as indicated. These two molecules are only examples for possible formats using IgG-CrossFab combinations which also were used for the bispecific molecules containing newly isolated DR5 binders. Other possibilities include the crossing in the IgG part of the molecule, the implementation of salt bridges and charged residues to stabilize the CrossFabs or the use of different linker lengths and sequences.

Figure 6: Comparison of aggregate contents during purification of different bispecific DR5-FAP antibody formats. All formats consist of Drozitumab IgG with a FAP binding domain (clone 4G8) fused to the C-terminus. FAP binding moieties consist of either a disulfide stabilized scFv (A), a

scFab (B) or a CrossFab (C). The chromatograms of the preparative size exclusion chromatography showed clear differences between the three constructs. While the production of the scFv and CrossFab molecules was comparable, the scFab containing construct showed lower yield and higher aggregate content. From this comparison the CrossFab containing molecule looked most promising.

Figure 7: Analysis of FACS binding on recombinant, human FAP expression HEK293 cells. DR5-FAP bispecific molecules in the new CrossFab format were compared to the scFab molecule, the parental 4G8 IgG and an unrelated negative control, Drozitumab. The two CrossFab molecules were shown to bind to FAP at least in the same range as the IgG control.

Figure 8: Surface plasmon resonance (SPR, Biacore) analysis of simultaneous binding of bispecific Drozitumab-FAP molecules (CrossFabs) to recombinant human DR5 and FAP. Biotinylated human DR5-Fc as the ligand was immobilized onto a streptavidin chip followed by injection of the first analyte (bispecific CrossFab molecules). After binding to DR5-Fc (90 sec association) and a short dissociation period (10 sec), recombinant soluble FAP (human or murine) in different concentrations (100 nM, 500 nM) was added as the second analyte and the additional response was measured. Binding of Drozitumab-X-FAP_A format (A and B) was compared to Drozitumab-X-FAP_B (C and D). Each construct was measured for binding DR5 and human (A, C) or murine FAP (B, D). By this analysis for all tested molecules a simultaneous binding to DR5 and human / murine FAP could be demonstrated.

Figure 9: DNA fragmentation ELISA assay for detection of apoptosis. Results of an apoptosis induction experiment in which two CrossFab molecules (Drozitumab-X-Fab-A and Drozitumab-X-Fab-B) were compared to Drozitumab and hyper-cross-linked Drozitumab in a two cell line (MDA-MB-231 and GM05389) co-culture assay. (A) Induction of apoptosis was detected after 24 hrs using a cell death detection ELISA. All tested constructs were used in concentrations of 7 nM and 0.7 nM. Apoptosis induction of target and effector cells is compared to apoptosis of target cells alone. (B) Comparison of co-culture bystander apoptosis induction in Cell Death Detection ELISA (DNA fragmentation) with Drozitumab (+/- Fc cross-linking) vs. bispecific DR5-FAP molecules consisting of a FAP CrossFab fused to the C-terminus of Drozitumab heavy chain. The molecule containing the affinity matured FAP binding moiety (28H1) shows superior activity compared to the molecule containing the lower affinity FAP binder.

Figure 10: DNA fragmentation ELISA assay for detection of apoptosis. Induction of apoptosis via bispecific Drozitumab-FAP CrossFab molecules is dependent on cross-linking via FAP. Figure 10 shows the results of apoptosis induction on MDA-MB-231 cells by CrossFab molecules cross-linked by recombinant human FAP coated to an ELISA plate. While the control molecule (Drozitumab cross-linked via anti Fc antibody) shows similar apoptosis induction, independent on coating of FAP or an unrelated control protein, the bispecific constructs only exhibited significant apoptosis activity when FAP was coated. With coating of the control plasmid only at highest construct concentration (7 nM) apoptosis could be detected which probably is due to the basal FAP expression of the MDA-MB-231 cell line. Apoptosis activity was similar for both tested CrossFab molecules and in the same range as observed with hyper-cross-linked Drozitumab.

Figure 11: Comparison of apoptosis induction (DNA fragmentation) on different human tumor cell lines in a co-culture experiment with FAP expressing human fibroblasts GM05389. Drozitumab and hyper-cross-linked Drozitumab is compared to a bispecific Drozitumab-4G8 CrossFab molecule (all at concentrations of 7.0 and 0.7 nM). Used tumor cell lines were MDA-MB-231 (breast cancer), U-87MG (glioblastoma), FaDu (squamous carcinoma) or A549 (lung carcinoma). Apoptosis induction at a concentration of 7 nM was similar for all four cell lines while at 0.7 nM a more pronounced difference between the cell lines was observed.

Figure 12: Schematic representation of the human DR5 antigen constructs used for isolation, screening and characterization of novel DR5 binders. For all constructs the same DR5 domain (extracellular domain, ECD; aa 56 – 207) was used either alone (A) as dimeric Fc fusion (B) or as monomeric Fc fusion (C) using the knob-into-hole technology. All antigens were transiently transfected and produced in HEK293 EBNA cells.

Figure 13: Screening of 46 unique DR5 binders (isolated by phage display) for induction of apoptosis on MDA-MB-231 cells (DNA fragmentation assay) after hyper-cross-linking with secondary anti Fc antibody. Antibodies were used at concentrations of 7 nM (black bars) and 0.7 nM (hatched bars). The vast majority of DR5 binders (42 / 46) were able to induce DNA fragmentation to different degrees after hyper-cross-linking.

Figure 14: Apoptosis induction in MDA-MB-231 cells measured via DNA fragmentation ELISA assay by a selected series of novel DR5 binders in the presence or absence of secondary, cross-linking anti Fc antibody to evaluate if the new DR5 antibodies exhibit apoptosis activity without secondary cross-linking. The new DR5 binders were compared to Drozitumab, an antibody known to be active to a certain degree without cross-linking.

Figure 15: Analysis of inhibition of cell proliferation (Cell TiterGlo Assay) of three different human tumor cells (DLD-1, NCI H460 and MDA-MB-231) upon treatment with different, cross-linked DR5 antibodies at a concentration of 7 nM.

Figure 16: Evaluation of apoptosis induction measured by Caspase 8 activation in three human tumor cell lines (DLD-1, NCI H460 and MDA-MB-231) after treatment with cross-linked DR5 antibodies at a concentration of 7 nM.

Figure 17: Results of epitope binning experiments by Surface Plasmon Resonance (Biacore). After binding of a first antibody to human DR5 further binding of additional antibodies to be tested was analyzed. With the exception of clone 422, which might overlap with the Drozitumab epitope, none of the tested new DR5 binders seem to bind to a region on DR5 that overlaps with Drozitumab epitope while among each other they might share at least overlapping epitopes.

Figure 18: TRAIL competition assay to determine ligand blocking vs. ligand non-blocking antibodies. Human TRAIL was immobilized on a CM5 chip. Then a complex consisting of human DR5-Fc and DR5 antibody was used as the analyte and binding of the complex to immobilized TRAIL was analyzed. While most of the new binders from phage display were TRAIL blocking molecules, a number of DR5 antibodies from rabbit immunization were of the non-blocking kind.

Figure 19: Effect of human TRAIL on inhibition of proliferation of DLD-1 target cells upon treatment with different DR5 agonistic antibodies. DLD-1 target cells were incubated with cross-linked DR5 antibodies in the absence (solid lines) or presence (stippled lines) of different concentrations of human TRAIL. Depending on the epitope recognized by the DR5 antibodies addition of TRAIL did (non-ligand blocking) or did not (ligand blocking) increase inhibition of cell growth. TRAIL alone and an antibody known to not block TRAIL were included as controls.

Figure 20: DNA fragmentation ELISA assay for detection of apoptosis. Apoptosis induction activity of different DR5-FAP bispecific antibodies (FAP clone 28H1) on MDA-MB-231 as the target cell line in the presence or absence of recombinant human FAP coated on a 96 well plate. In this setting the coated FAP should mimic FAP expressed in the tumor stroma. Bispecific antibodies were tested at two concentrations (7.0 and 0.7 nM). For the bispecific molecules containing the novel DR5 binders a concentration dependent induction of apoptosis only was detected in the presence of FAP whereas the control molecule containing Drozitumab also showed some activity in the absence of FAP, confirming that the activity of the new DR5 antibodies is strictly dependent on cross-linking.

Figure 21: Bystander apoptosis induction activity of bispecific DR5-FAP molecules (2+2 CrossFab format) in a co-culture assay using MDA-MB-231 as DR5 expressing target cells and GM05389 (FAP^+ fibroblasts) for cross-linking. Molecules containing different newly isolated DR5 binders were compared to Drozitumab containing constructs at three concentrations (7.0, 0.7 and 0.07 nM). Under these conditions not all tested bispecific constructs exhibited the same activity compared to cross-linking via anti Fc mAb or via recombinant FAP. This shows that not every antibody that can principally induce apoptosis after cross-linking also would do that under more natural conditions (in which the antigens-DR5 and FAP-are expressed on different cell types).

Figure 22: Cell death detection ELISA (DNA fragmentation) with MDA-MB-231 target cells treated with new DR5 binding Fabs fused to the C-terminus of human Fc, compared to Drozitumab, all after cross-linking with anti Fc antibody. All tested Fc fusion molecules are able to confer apoptosis induction on target cells in the same range as cross-linked Drozitumab, indicating that their binding and activity capacity is not blocked by positioning of the Fc to their N-terminus. A: DR5 binders derived from phage display B: Humanized DR5 binders derived from immunization.

Figure 23: Comparison of bispecific DR5-FAP antibodies (new DR5 binders with C-terminal 28H1 CrossFab fusion) for apoptosis induction on MDA-MB-231 as the target cell line in a co-culture assay using GM05389 fibroblasts (DNA fragmentation ELISA assay for detection of apoptosis).

Figure 24: Bispecific DR5-FAP antibodies induce apoptosis on G401 cells in a bystander assay as determined by DNA fragmentation in a Cell Death Detection ELISA.

Figure 25: Schematic representation of the additional bispecific DR5-FAP CrossFab formats that were evaluated with respect to productivity and quality. These four molecules differ in the position and valency of DR5 and FAP binding moieties: two 2+1 molecules, one 3+1 and one

1+1 construct were evaluated. One 2+1 molecule contains one FAP (28H1) CrossFab fused to the C-terminus of a DR5 (5E11) heavy chain (A) whereas a second 2+1 format consists of two DR5 (5E11) Fabs fused to the N-terminus of an Fc with the 28H1 CrossMab counterpart (B). The third molecule (C) contains three DR5 targeting Fabs and one 28H1 CrossFab fused at the C-terminus of a heavy chain. In the 1+1 format (D) the DR5 binder #174 is combined with the FAP binding moiety 4B9 in CrossFab arrangement. All constructs depend on hetero-dimerization using the knob-into-hole technology.

Figure 26: Apoptosis activity of bispecific DR5-FAP molecules (A: 5E11-28H1; B: 18F11-28H1) in different formats as measured by Cell Death Detection ELISA for DNA fragmentation in MDA-MB-231 cells co-cultured with GM05489 fibroblasts. The standard 2+2 format of Drozitumab-28H1 was compared to the 5E11-28H1 and 18F11-28H1 molecules in 2+2, 2+1 and 3+1 formats at three different concentrations.

Figure 27: Bystander apoptosis induction of the 5E11-28H1 molecule (2+2 format) compared to three additional bispecific formats: 2+1, novel 2+1 (head-to-tail fusion) and 3+1. MDA-MB-231 were used as target cells, GM05389 fibroblasts were co-cultured for FAP dependent cross-linking and the bispecific antibodies were evaluated at concentrations from 700 -0.007 nM.

Figure 28: New bispecific 5E11-28H1 formats (2+2) for evaluation of productivity, side product profile and activity. The molecules differ in site and type of crossing.

A: C-terminal crossing of 28H1 (CH1CL) B: Crossing of entire N-terminal 5E11 Fab
C: N-terminal VHVL crossing of 5E11 D: N-terminal CH1CL crossing of 5E11
E: Complete crossing of C-terminal 28H1 Fab F: Crossing of C-terminal 28H1 Fab (VHVL)

Figure 29: FACS binding results with 5E11-28H1 CrossFabs in three different formats on MDA-MB-231 over a concentration range from 0.0037 to 60 nM. A PE conjugated goat-anti-human Fc $(Fab)_2$ was used for detection.

Figure 30: DNA fragmentation ELISA assay for detection of apoptosis. Induction of apoptosis of 4 different CrossMab variants in co- (A) and mono-culture (B, C) settings as detected by DNA fragmentation. All 4 different variants induce apoptosis in tumor cells in the co-culture setting in a comparable dose-dependent manner. In mono-culture settings no apoptosis is induced neither in MDA-MB231 tumor cell line nor in GM05389 fibroblast cell line, pointing out the specificity and FAP-dependency of apoptosis induction of all 4 variants.

Figure 31: *In vivo* efficacy in mouse xenograft tumor models in which DLD-1 (A; nude mice) or MDA-MB-231 (B; SCID-beige mice) cells were co-injected with mu FAP expressing 3T3 fibroblasts to ensure FAP expression in the tumor stroma. After the tumors have been engrafted treatment at 10 mg/kg was performed by i.v. injection of the bispecific Drozitumab-28H1 molecule (triangle) or Drozitumab alone (square) or the vehicle control diamond). Efficacy was determined by means of tumor growth inhibition (TGI) compared to the vehicle control.

Figure 32: Evaluation of bispecific DR5-FAP molecules produced for *in vivo* efficacy experiments for their apoptosis induction activity. The four different molecules were tested in a Cell Death Detection ELISA for DNA fragmentation of DLD-1 cells. 3T3 or recombinant 3T3

cells expressing murine FAP wereused in the co-culture assay for cross-linking. Fold increase of apoptosis in comparison to untreated cells are shown.

Figure 33: Viability of DLD-1 cells after treatment with DR5-FAP bispecific antibodies after co-culture assay with 3T3 cells or 3T3 cells expressing murine FAP (Cell TiterGlo). Percentage of viability compared to an untreated control is given.

Figure 34: Mouse xenograft models in which different DR5-FAP bispecific molecules (10 mg/kg) in the 2+2 format were compared to a vehicle control. All constructs contained the 28H1 FAP CrossFab fused to different DR5 binders: 5E11, 174 and 422. In addition one molecule (5E11-28H1) was included in which the Fc carried mutations to inhibit any FcγR interaction (PGLALA). Efficacy was determined as tumor growth inhibition (TGI).

Figure 35: DNA fragmentation assay. Anti-DR5 antibodies induce cell death upon receptor hyperclustering in a dose-dependent manner. The generated rabbit anti-DR5 antibodies were able to induce apoptosis of MDA-MB231 cells with different potencies but always in a dose-dependent fashion and only after Fc-mediated cross-linking of the DR5 molecules (upper panel). In the absence of an anti-rabbit Fc-specific secondary antibody, no significant cell death was detected (lower panel).

Figure 36: Cell viability is diminished by anti-DR5 antibodies upon receptor hyperclustering in a dose-dependent manner. The generated rabbit anti-DR5 antibodies were able to decrease the viability of MDA-MB231 cells with varying potencies but always in a dose-dependent fashion and only after Fc-mediated cross-linking of the DR5 molecules (upper panel). In the absence of an anti-rabbit Fc-specific secondary antibody, the cell viability was not affected at any antibody concentration (lower panel).

Figure 37: Analysis of inhibition of cell proliferation (Cell TiterGlo Assay) of three different human tumor cells (DLD-1, NCI H460 and MDA-MB-231) upon treatment with different, cross-linked DR5 antibodies at a concentration of 7 nM.

Figure 38: Evaluation of apoptosis induction measured by Caspase 8 activation in three human tumor cell lines (DLD-1, NCI H460 and MDA-MB-231) after treatment with cross-linked DR5 antibodies at a concentration of 7 nM.

Figure 39: Relative active concentration of stressed DR5 antibody samples: Exemplary response curve.

Figure 40: Relative active concentrations of original and stressed DR5 antibodies derived from immunization.

Figure 41: Relative active concentrations of original and stressed DR5 antibodies derived from phage display.

Figure 42: Surface plasmon resonance (SPR, Biacore) analysis of simultaneous binding of different DR5-FAP bispecific antibodies to both recombinant targets. In a first reaction binding of the bispecific antibodies to recombinant human DR5-Fc was determined, followed by analysis of binding to recombinant human (upper panel)) or murine FAP (lower panel).

Figure 43: Induction of apoptosis in DLD-1 and H460 tumor cell lines by 2+2 bispecific constructs in co-culture assays as detected by DNA fragmentation. While a bispecific construct containing Drozitumab as DR5-binding component already induces apoptosis in the absence of FAP, all constructs containing new DR5 binders derived by immunization only induce apoptosis in the presence of FAP. Constructs with newly developed DR5 binders, such as 0011-28H1 and 0016-28H1, are able to induce apoptosis to a higher extent especially at low concentrations as compared to the Drozitumab containing bispecific construct.

Figure 44: Binding of anti-DR5-FAP bispecific 2+2 constructs to the DR5-expressing tumor cell line MDA-MB-231 as measured by flow cytometry analysis.

Figure 45: A Induction of apoptosis of humanized variants after crosslinking as detected by DNA fragmentation (Cell Death Detection ELISA): Humanized Variants of DR5TAA-0011 (DR5TAA-0066 – DR5TAA-0075, black lines) induce apoptosis upon crosslinking with secondary antibody in a dose-dependent manner. Several humanized variants, such as DR5TAA-0067, DR5TAA-0071, DR5TAA-0074 and DR5TAA-0075, are able to induce apoptosis in a similar manner concerning maximum of induction and dose-dependency as compared to the chimeric variant (DR5TAA-0052, grey lines). **B** Absence of induction of apoptosis of humanized variants without additional crosslinking as detected by DNA fragmentation (Cell Death Detection ELISA): Humanized Variants of DR5TAA-0011 (DR5TAA-0066 – DR5TAA-0075) induce no apoptosis if not crosslinked by a secondary antibody.

Figure 46: Comparable induction of cell death in a co-culture system by bispecific anti-DR5-FAP antibodies in 1+1 and 2+2 formats.

Figure 47: Great specificity of bispecific anti-DR5-FAP antibodies at inducing cell death only in the presence of both tumor cells and fibroblasts.

Figure 48: Efficacy of bispecific anti-DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) in DLD-1 CRC co-injection cell line based xenograft model

Figure 49: Efficacy of bispecific anti-DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) in Co5896 CRC fragment based patient derived xenograft model (PDX)

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

"Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

The term "A bispecific antibody that specifically binds death receptor 5 (DR5) and Fibroblast Activation Protein (FAP)" refers to a bispecific antibody that is capable of binding DR5 and FAP with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting cells expressing DR5 and FAP). Specifically "A bispecific antibody that specifically binds death receptor 5 (DR5) and Fibroblast Activation Protein (FAP)" refers to a bispecific antibody targeting DR5 on a tumor cell and FAP in the stroma surrounding said tumor. In one embodiment, the extent of binding of a bispecific antibody that specifically binds death receptor 5 (DR5) and Fibroblast Activation Protein (FAP) to an unrelated, non-FAP

or non-DR5 protein is less than about 10% of the binding of the antibody to DR5 or FAP as measured, e.g., by a Enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR) based assays (e.g. Biacore) or flow cytometry (FACS). In certain embodiments, a bispecific antibody that specifically binds death receptor 5 (DR5) and Fibroblast Activation Protein (FAP) has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, a bispecific antibody that specifically binds death receptor 5 (DR5) and Fibroblast Activation Protein (FAP) binds to an epitope of DR5 or FAP that is conserved among DR5 or FAP from different species. Preferably said bispecific antibody binds to human and cynomolgous monkey DR5 and to human, cynomolgous monkey and mouse FAP.

The terms “An antibody that specifically binds death receptor 5 (DR5)” refers to an antibody that is capable of binding DR5 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting cells expressing DR5. In one embodiment, the extent of binding of an antibody that specifically binds death receptor 5 (DR5) to an unrelated non-DR5 protein is less than about 10% of the binding of the antibody to DR5 as measured, e.g., by a radioimmunoassay (RIA) or flow cytometry (FACS). In certain embodiments, an antibody that specifically binds death receptor 5 (DR5) has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, $\leq$ 0.1 nM, $\leq$ 0.01 nM, or $\leq$ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an antibody that specifically binds death receptor 5 (DR5) binds to an epitope of DR5 that is conserved among DR5 from different species. Preferably said antibody binds to human and cynomolgous monkey DR5. The term “An antibody that specifically binds death receptor 5 (DR5)” also encompasses bispecific antibodies that are capable of binding DR5 and a second antigen.

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

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab’-SH, $F(ab')_2$; diabodies, cross-Fab fragments; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. scFv antibodies are, e.g. described

in Houston, J.S., Methods in Enzymol. 203 (1991) 46-96). In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely being able to assemble together with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

As used herein, "Fab fragment" refers to an antibody fragment comprising a light chain fragment comprising a VL domain and a constant domain of a light chain (CL), and a VH domain and a first constant domain (CH1) of a heavy chain. In one embodiment the bispecific antibodies of the invention comprise at least one Fab fragment, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged. Due to the exchange of either the variable regions or the constant regions, said Fab fragment is also referred to as "cross-Fab fragment" or "xFab fragment" or "crossover Fab fragment". Two different chain compositions of a crossover Fab molecule are possible and comprised in the bispecific antibodies of the invention: On the one hand, the variable regions of the Fab heavy and light chain are exchanged, i.e. the crossover Fab molecule comprises a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab molecule is also referred to as CrossFab $_{(VLVH)}$. On the other hand, when the constant regions of the Fab heavy and light chain are exchanged, the crossover Fab molecule comprises a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). This crossover Fab molecule is also referred to as CrossFab $_{(CLCH1)}$.

A "single chain Fab fragment" or "scFab" is a polypeptide consisting of an antibody heavy chain variable domain (VH), an antibody constant domain 1 (CH1), an antibody light chain variable domain (VL), an antibody light chain constant domain (CL) and a linker, wherein said antibody domains and said linker have one of the following orders in N-terminal to C-terminal direction: a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 or d) VL-CH1-linker-VH-CL; and wherein said linker is a polypeptide of at least 30 amino acids, preferably between 32 and 50 amino acids. Said single chain Fab fragments a) VH-CH1-linker-VL-CL, b) VL-CL-linker-VH-CH1, c) VH-CL-linker-VL-CH1 and d) VL-CH1-linker-VH-CL, are stabilized via the natural disulfide bond between the CL domain and the CH1 domain. In addition, these single chain Fab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (e.g. position 44 in the variable heavy chain and positionn 100 in the variable light chain according to Kabat numbering). The term "N-terminus

denotes the last amino acid of the N-terminus. The term “C-terminus denotes the last amino acid of the C-terminus.

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

The term “linker” as used herein refers to a peptide linker and is preferably a peptide with an amino acid sequence with a length of at least 5 amino acids, preferably with a length of 5 to 100, more preferably of 10 to 50 amino acids. In one embodiment said peptide linker is (GxS)n or (GxS)nGm with G = glycine, S = serine, and (x = 3, n= 3, 4, 5 or 6, and m= 0, 1, 2 or 3) or (x = 4,n= 2, 3, 4 or 5 and m= 0, 1, 2 or 3), preferably x = 4 and n= 2 or 3, more preferably with x = 4, n= 2. In one embodiment said peptide linker is $(G_4S)_2$.

The term “immunoglobulin molecule” refers to a protein having the structure of a naturally occurring antibody. For example, immunoglobulins of the IgG class are heterotetrameric glycoproteins of about 150,000 daltons, composed of two light chains and two heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3), also called a heavy chain constant region. Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain, also called a light chain constant region. The heavy chain of an immunoglobulin may be assigned to one of five types, called α (IgA), δ (IgD), ε (IgE), γ (IgG), or μ (IgM), some of which may be further divided into subtypes, e.g. γ_1 (IgG_1), γ_2 (IgG_2), γ_3 (IgG_3), γ_4 (IgG_4), α_1 (IgA_1) and α_2 (IgA_2). The light chain of an immunoglobulin may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain. An immunoglobulin essentially consists of two Fab molecules and an Fc domain, linked via the immunoglobulin hinge region.

An “antibody that binds to the same epitope” as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

The term "antigen binding domain" refers to the part of an antigen binding molecule that comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antigen binding molecule may only bind to a particular part of the

antigen, which part is termed an epitope. An antigen binding domain may be provided by, for example, one or more antibody variable domains (also called antibody variable regions). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species, usually prepared by recombinant DNA techniques. Chimeric antibodies comprising a rabbit variable region and a human constant region are preferred. Other preferred forms of "chimeric antibodies" encompassed by the present invention are those in which the constant region has been modified or changed from that of the original antibody to generate the properties according to the invention, especially in regard to C1q binding and/or Fc receptor (FcR) binding. Such chimeric antibodies are also referred to as "class-switched antibodies". Chimeric antibodies are the product of expressed immunoglobulin genes comprising DNA segments encoding immunoglobulin variable regions and DNA segments encoding immunoglobulin constant regions. Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art. See e.g. Morrison, S.L., et al., Proc. Natl. Acad. Sci. USA 81 (1984) 6851-6855; US Patent Nos. 5,202,238 and 5,204,244.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

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

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

The term “amino acid mutation” as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., reduced binding to an Fc receptor, or increased association with another peptide. Amino acid sequence deletions and insertions include amino- and/or carboxy-terminal deletions and insertions of amino acids. Particular amino acid mutations are amino acid substitutions. For the purpose of altering e.g. the binding characteristics of an Fc region, non-conservative amino acid substitutions, i.e. replacing one amino acid with another amino acid having different structural and/or chemical properties, are particularly preferred. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids (e.g. 4-hydroxyproline, 3-methylhistidine, ornithine, homoserine, 5-hydroxylysine). Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid mutation. For example, a substitution from proline at position 329 of the Fc domain to glycine can be indicated as 329G, G329, G_{329}, P329G, or Pro329Gly.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term “Fc domain” or “Fc region” herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region is usually defined to extend from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present.

Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991. A "subunit" of an Fc domain as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

A "modification promoting the association of the first and the second subunit of the Fc domain" is a manipulation of the peptide backbone or the post-translational modifications of an Fc domain subunit that reduces or prevents the association of a polypeptide comprising the Fc domain subunit with an identical polypeptide to form a homodimer. A modification promoting association as used herein particularly includes separate modifications made to each of the two Fc domain subunits desired to associate (i.e. the first and the second subunit of the Fc domain), wherein the modifications are complementary to each other so as to promote association of the two Fc domain subunits. For example, a modification promoting association may alter the structure or charge of one or both of the Fc domain subunits so as to make their association sterically or electrostatically favorable, respectively. Thus, (hetero)dimerization occurs between a polypeptide comprising the first Fc domain subunit and a polypeptide comprising the second Fc domain subunit, which might be non-identical in the sense that further components fused to each of the subunits (e.g. antigen binding moieties) are not the same. In some embodiments the modification promoting association comprises an amino acid mutation in the Fc domain, specifically an amino acid substitution. In a particular embodiment, the modification promoting association comprises a separate amino acid mutation, specifically an amino acid substitution, in each of the two subunits of the Fc domain.

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

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. As also mentioned for chimeric and humanized antibodies according to the invention the term “human antibody” as used herein also comprises such antibodies which are modified in the constant region to generate the properties according to the invention, especially in regard to C1q binding and/or FcR binding, e.g. by “class switching” i.e. change or mutation of Fc parts (e.g. from IgG1 to IgG4 and/or IgG1/IgG4 mutation.)

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from a host cell such as a NS0 or CHO cell or from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes or antibodies expressed using a recombinant expression vector transfected into a host cell. Such recombinant human antibodies have variable and constant regions in a rearranged form. The recombinant human antibodies according to the invention have been subjected to in vivo somatic hypermutation. Thus, the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germ line VH and VL sequences, may not naturally exist within the human antibody germ line repertoire in vivo.

A “human consensus framework” is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is

subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra.*

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

The term "hypervariable region" or "HVR," as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) Hypervariable regions (HVRs) are also referred to as complementarity determining regions (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof

is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table A as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

TABLE A. CDR Definitions[1]

CDR	Kabat	Chothia	AbM[2]
V_H CDR1	31-35	26-32	26-35
V_H CDR2	50-65	52-58	50-58
V_H CDR3	95-102	95-102	95-102
V_L CDR1	24-34	26-32	24-34
V_L CDR2	50-56	50-52	50-56
V_L CDR3	89-97	91-96	89-97

[1] Numbering of all CDR definitions in Table A is according to the numbering conventions set forth by Kabat et al. (see below).

[2] "AbM" with a lowercase "b" as used in Table A refers to the CDRs as defined by Oxford Molecular's "AbM" antibody modeling software.

Kabat *et al.* also defined a numbering system for variable region sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable region sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody variable region are according to the Kabat numbering system.

With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra.*

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

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

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

“Isolated nucleic acid encoding a bispecific antibody that specifically binds DR5 and FAP antibody” refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and

methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A “naked antibody” refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

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

“No substantial cross-reactivity” means that a molecule (*e.g.*, an antibody) does not recognize or specifically bind an antigen different from the actual target antigen of the molecule (e.g. an antigen closely related to the target antigen), particularly when compared to that target antigen. For example, an antibody may bind less than about 10% to less than about 5% to an antigen different from the actual target antigen, or may bind said antigen different from the actual target antigen at an amount consisting of less than about 10%, 9%, 8% 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.2%, or 0.1%, preferably less than about 2%, 1%, or 0.5%, and most preferably less than about 0.2% or 0.1% antigen different from the actual target antigen.

“Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment

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

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

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

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

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

The term "death receptor 5 (DR5)", as used herein, refers to any native DR5 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed DR5 as well as any form of DR5 that results from processing in the cell. The term also encompasses naturally occurring variants of DR5, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human DR5 is shown in SEQ ID NO.:155.

The term "Fibroblast activation protein (FAP)", as used herein, refers to any native FAP from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed FAP as well as any form of FAP that results from processing in the cell. The term also encompasses naturally occurring variants of FAP, e.g., splice variants or allelic variants. Preferably, an anti-FAP antibody of the invention binds to the extracellular domain of FAP. The amino acid sequence of exemplary human, mouse and cynomolgus monkey FAP ectodomains (with a C-terminal poly-lysine and 6x His-tag) are shown in SEQ ID NO.:156, SEQ ID NO.:157, and SEQ ID NO.:158 respectively.

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

The term cancer as used herein refers to proliferative diseases, such as lymphomas, lymphocytic leukemias, lung cancer, non small cell lung (NSCL) cancer, bronchioloalviolar cell lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, gastric cancer, colon cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the

vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, prostate cancer, cancer of the bladder, cancer of the kidney or ureter, renal cell carcinoma, carcinoma of the renal pelvis, mesothelioma, hepatocellular cancer, biliary cancer, neoplasms of the central nervous system (CNS), spinal axis tumors, brain stem glioma, glioblastoma multiforme, astrocytomas, schwanomas, ependymonas, medulloblastomas, meningiomas, squamous cell carcinomas, pituitary adenoma and Ewings sarcoma, including refractory versions of any of the above cancers, or a combination of one or more of the above cancers.

The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

The term "antigen-binding site of an antibody" when used herein refer to the amino acid residues of an antibody which are responsible for antigen-binding. The antigen-binding portion of an antibody comprises amino acid residues from the "complementary determining regions" or "CDRs". "Framework" or "FR" regions are those variable domain regions other than the hypervariable region residues as herein defined. Therefore, the light and heavy chain variable domains of an antibody comprise from N- to C-terminus the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Especially, CDR3 of the heavy chain is the region which contributes most to antigen binding and defines the antibody's properties. CDR and FR regions are determined according to the standard definition of Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991) and/or those residues from a "hypervariable loop".

Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. "Bispecific antibodies" according to the invention are antibodies which have two different antigen-binding specificities.

Antibodies of the present invention are specific for two different antigens, i.e. DR5 as first antigen and FAP as second antigen.

The term "monospecific" antibody as used herein denotes an antibody that has one or more binding sites each of which bind to the same epitope of the same antigen.

The term "bispecific" antibody as used herein denotes an antibody that has at least two binding sites each of which bind to different epitopes of the same antigen or a different antigen.

The antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. Provided herein is a bispecific antibody, with binding specificities for FAP and DR5. In certain embodiments, bispecific antibodies may bind to two different epitopes of DR5. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express DR5. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature* 305: 537 (1983)), WO 93/08829, and Traunecker et al., *EMBO J.* 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,e.g. Gruber et al., *J. Immunol.*, 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.* 147: 60 (1991).

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising at least one antigen binding site that binds to FAP or DR5 as well as another, different antigen (see, US 2008/0069820, for example).

The term "valent" as used within the current application denotes the presence of a specified number of binding sites in an antibody molecule. As such, the terms "bivalent", "tetravalent", and "hexavalent" denote the presence of two binding sites, four binding sites, and

six binding sites, respectively, in an antibody molecule. The bispecific antibodies according to the invention are at least “bivalent” and may be “trivalent” or “multivalent” (e.g.“tetravalent” or “hexavalent”).

Antibodies of the present invention have two or more binding sites and are bispecific. That is, the antibodies may be bispecific even in cases where there are more than two binding sites (i.e. that the antibody is trivalent or multivalent). Bispecific antibodies of the invention include, for example, multivalent single chain antibodies, diabodies and triabodies, as well as antibodies having the constant domain structure of full length antibodies to which further antigen-binding sites (e.g., single chain Fv, a VH domain and/or a VL domain, Fab, or (Fab)2) are linked via one or more peptide-linkers. The antibodies can be full length from a single species, or be chimerized or humanized.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

The term ”amino acid” as used within this application denotes the group of naturally occurring carboxy α-amino acids comprising alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gln, Q), glutamic acid (glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

As used herein, the expressions “cell”, “cell line”, and “cell culture” are used interchangeably and all such designations include progeny. Thus, the words “transfectants” and “transfected cells” include the primary subject cell and cultures derived there from without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological activity as screened for in the originally transformed cell are included.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity

which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

As used herein, the term “binding” or “specifically binding” refers to the binding of the antibody to an epitope of the antigen in an in-vitro assay, preferably in a surface plasmon resonance assay (SPR, BIAcore, GE-Healthcare Uppsala, Sweden). The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), kD (dissociation constant), and KD (kD/ka). Binding or specifically binding means a binding affinity (KD) of 10-8 mol/l or less, preferably 10-9 M to 10-13 mol/l.

Binding of the antibody to the death receptor can be investigated by a BIAcore assay (GE-Healthcare Uppsala, Sweden). The affinity of the binding is defined by the terms ka (rate constant for the association of the antibody from the antibody/antigen complex), kD (dissociation constant), and KD (kD/ka)

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinant include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody.

As used herein, the terms “engineer, engineered, engineering,” particularly with the prefix “glyco-,” as well as the term “glycosylation engineering” are considered to include any manipulation of the glycosylation pattern of a naturally occurring or recombinant polypeptide or fragment thereof. Glycosylation engineering includes metabolic engineering of the glycosylation machinery of a cell, including genetic manipulations of the oligosaccharide synthesis pathways to achieve altered glycosylation of glycoproteins expressed in cells. Furthermore, glycosylation engineering includes the effects of mutations and cell environment on glycosylation. In one embodiment, the glycosylation engineering is an alteration in glycosyltransferase activity. In a particular embodiment, the engineering results in altered glucosaminyltransferase activity and/or fucosyltransferase activity.

II. COMPOSITIONS AND METHODS

In one aspect, the invention is based on bispecific antibodies comprising a first antigen binding site specific for TRAIL death receptor 5 (DR5) and a second antigen binding site specific for Fibroblast Activation Protein (FAP). In another embodiment novel antibodies targeting DR5 are provided. Antibodies of the invention are useful, e.g., for the treatment or diagnosis of cancer.

A. Exemplary bispecific antibodies that bind to DR5 and FAP

In one aspect, the invention provides isolated bispecific antibodies that bind to DR5 and FAP. FAP binding moieties have been described in WO 2012/020006, which is included by reference in its entirety. FAP binding moieties of particular interest to be used in the DR5-FAP bispecific antibodies are outlined in the embodiments below.

In certain embodiments, a bispecific antibody that binds to DR5 and FAP specifically crosslinks the death receptors and apoptosis of the target 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 outlined above the inventors of the present invention developed novel DR5 binding moieties with superior properties compared to known DR5 binders that can be incorporated into novel and advantageous DR5-FAP bispecific antibodies.

In one aspect, the 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 invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, comprising

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

(b) a heavy chain CDR2 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 at least one antigen binding site specific for FAP, comprising

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, 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

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:25;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:25;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:27;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:27;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:25;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:25;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:75;

(b) a heavy chain CDR2 of SEQ ID NO.:83;

(c) a heavy chain CDR3 of SEQ ID NO.:84;

(d) a light chain CDR1 of SEQ ID NO.:86;

(e) a light chain CDR2 of SEQ ID NO.:28;

(f) a light chain CDR3 of SEQ ID NO.:87

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:75;

(b) a heavy chain CDR2 of SEQ ID NO.:83;

(c) a heavy chain CDR3 of SEQ ID NO.:84;

(d) a light chain CDR1 of SEQ ID NO.:86;

(e) a light chain CDR2 of SEQ ID NO.:28;

(f) a light chain CDR3 of SEQ ID NO.:87

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, 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.:96;

(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.:99;

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, 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.:96;

(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.:99;

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising at least one antigen binding site specific for DR5, 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.:104;

(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:105;

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, 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.:104;

(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:105;

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, 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.:108;

(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:109;

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, 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.:108;

(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:109;

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

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

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

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

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

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

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, 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.:98;

(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.:97;

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

(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;

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

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising

at least one antigen binding site specific for DR5, 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.:98;
(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.:97;
and at least one antigen binding site specific for FAP, comprising
(a) a heavy chain CDR1 of SEQ ID NO.:33;
(b) a heavy chain CDR2 of SEQ ID NO.:34;
(c) a heavy chain CDR3 of SEQ ID NO.:35;
(d) a light chain CDR1 of SEQ ID NO.:36;
(e) a light chain CDR2 of SEQ ID NO.:37;
(f) a light chain CDR3 of SEQ ID NO.:38.

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising
at least one antigen binding site specific for DR5, comprising
(a) a heavy chain CDR1 of SEQ ID NO.:17;
(b) a heavy chain CDR2 of SEQ ID NO.:25;
(c) a heavy chain CDR3 of SEQ ID NO.:19;
(d) a light chain CDR1 of SEQ ID NO.:27;
(e) a light chain CDR2 of SEQ ID NO.:28;
(f) a light chain CDR3 of SEQ ID NO.:22;
and at least one antigen binding site specific for FAP, comprising
(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;
(f) a light chain CDR3 of SEQ ID NO.:14.

In one aspect, the invention provides a bispecific antibody that binds to DR5 and FAP comprising
at least one antigen binding site specific for DR5, comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;
(b) a heavy chain CDR2 of SEQ ID NO.:25;
(c) a heavy chain CDR3 of SEQ ID NO.:19;
(d) a light chain CDR1 of SEQ ID NO.:27;
(e) a light chain CDR2 of SEQ ID NO.:28;
(f) a light chain CDR3 of SEQ ID NO.:22;

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

(a) a heavy chain CDR1 of SEQ ID NO.:33;
(b) a heavy chain CDR2 of SEQ ID NO.:34;
(c) a heavy chain CDR3 of SEQ ID NO.:35;
(d) a light chain CDR1 of SEQ ID NO.:36;
(e) a light chain CDR2 of SEQ ID NO.:37;
(f) a light chain CDR3 of SEQ ID NO.:38.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5 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;

and at least one antigen binding site specific for FAP comprisings a variable heavy chain comprising an amino acid sequence selected from the group of: SEQ ID NO.:15 and SEQ ID NO.:39 ; and a light chain variable region comprising an amino acid sequence selected from the group of SEQ ID NO.:16 and SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, 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; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region

comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, 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; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:24; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:24; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:24; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ

ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:24; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:29; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:29; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:30; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:30; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:31; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:31; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:32; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:32; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:30; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region

comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:30; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:31; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:31; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:82 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:85; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ

ID NO.:82 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:85; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:100 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:101; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:100 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:101; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:102 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:103; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:102 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:103; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:106 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:107; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:106 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:107; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:94 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:95; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

In one embodiment, the bispecific antibody comprises at least one antigen binding site specific for DR5, comprising a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:94 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:95; and at least one antigen binding site specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:39 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:40.

In one embodiment, the bispecific antibody of the invention comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO.:151.

In one embodiment, the bispecific antibody of the invention comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO.:152.

In another embodiment the bispecific antibody of the invention comprises a light chain constant region comprising the amino acid sequence of SEQ ID NO.:153.

In one embodiment, the bispecific antibody of the invention comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO.:151, wherein the C-terminal Lysine has been removed.

In one embodiment, the bispecific antibody of the invention comprises a heavy chain constant region comprising the amino acid sequence of SEQ ID NO.:152, wherein the C-terminal Lysine has been removed.

In one embodiment the bispecific antibody of the invention comprises a first antibody comprising at least one antigen binding site specific for DR5, said first antibody comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8, and a heavy chain constant region comprising the amino acid sequence selected from of SEQ ID NO.:151 or SEQ ID NO.:152, and a light chain constant region comprising the amino acid sequence of SEQ ID NO.:153, and a second antibody specific for FAP comprising one or more amino acid sequences as defined in any of the embodiments above. In one embodiment the C-terminal Lysine of the amino acid sequence of said heavy chain constant region has been removed.

In one embodiment the bispecific antibody of the invention comprises a first antibody comprising at least one antigen binding site specific for DR5, said first antibody comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8, and a heavy chain constant region comprising the amino acid sequence selected from of SEQ ID NO.:151 or SEQ ID NO.:152, and a light chain constant region comprising the amino acid sequence of SEQ ID NO.:153, and a second antibody specific for FAP comprising a variable heavy chain of SEQ ID NO.:15 and a variable light chain of SEQ ID NO.:16.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:131, SEQ ID NO.:132 and SEQ ID NO.:124.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:133, SEQ ID NO.:132 and SEQ ID NO.:124.

In one preferred embodiment a bispecific antibody is provided comprising SEQ ID NO.:134, SEQ ID NO.:132 and SEQ ID NO.:124.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:262, SEQ ID NO.:263 and SEQ ID NO.:132.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:135, SEQ ID NO.:136 and SEQ ID NO.:137.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:138, SEQ ID NO.:139 and SEQ ID NO.:137.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:274, SEQ ID NO.:275, and SEQ ID NO.:137.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:276, SEQ ID NO.:277, and SEQ ID NO.:132.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:278, SEQ ID NO.:279 and SEQ ID NO.:132.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:142, SEQ ID NO.:143, SEQ ID NO.:124 and SEQ ID NO.:132.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:145, SEQ ID NO.:146, SEQ ID NO.:124 and SEQ ID NO.:132.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:144, SEQ ID NO.:143, SEQ ID NO.:124 and SEQ ID NO.:132.

In one embodiment a bispecific antibody is provided comprising SEQ ID NO.:159, SEQ ID NO.:160, SEQ ID NO.:161 and SEQ ID NO.:162.

In another aspect, a bispecific antibody that binds to DR5 and FAP comprises at least one antigen binding site specific for DR5 comprising a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.:7, and at least one antigen binding site specific for FAP comprising a variable heavy chain of SEQ ID NO.:15 and a variable light chain of SEQ ID NO.:16.

In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions),

insertions, or deletions relative to the reference sequence, but a bispecific antibody that binds to DR5 and FAP comprising that sequence retains the ability to bind to FAP and DR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.:7. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the bispecific antibody that binds to DR5 and FAP comprises the VH sequence in SEQ ID NO.:7, including post-translational modifications of that sequence.

In another aspect, a bispecific antibody that binds to DR5 and FAP comprises at least one antigen binding site specific for DR5 comprising a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.:8, and at least one antigen binding site specific for FAP comprising a variable heavy chain of SEQ ID NO.:15 and a variable light chain of SEQ ID NO.:16.

In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but a bispecific antibody that binds to DR5 and FAP comprising that sequence retains the ability to bind to DR5 and FAP. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.:8. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the bispecific antibody that binds to DR5 and FAP comprises the VL sequence in SEQ ID NO:8, including post-translational modifications of that sequence.

In another aspect, a bispecific antibody that binds to DR5 and FAP is provided, comprising at least one antigen binding site specific for DR5 comprising a variable light chain of SEQ ID NO.:8 and a variable heavy chain of SEQ ID NO.:7; and at least one antigen binding site specific for FAP, comprising a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.:15. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but a bispecific antibody that binds to DR5 and FAP comprising that sequence retains the ability to bind to FAP and DR5. In certain embodiments, a total of 1 to 10 amino acids have been

substituted, inserted and/or deleted in SEQ ID NO.:15. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the bispecific antibody that binds to DR5 and FAP comprises the VH sequence in SEQ ID NO.:15, including post-translational modifications of that sequence.

In another aspect, a bispecific antibody that binds to DR5 and FAP is provided, comprising at least one antigen binding site specific for DR5, comprising a variable light chain of SEQ ID NO.:8 and a variable heavy chain of SEQ ID NO.:7, and at least one antigen binding site specific for FAP, comprising a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.:16. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but a bispecific antibody that binds to DR5 and FAP comprising that sequence retains the ability to bind to DR5 and FAP. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.:16. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the bispecific antibody that binds to DR5 and FAP comprises the VL sequence in SEQ ID NO:16, including post-translational modifications of that sequence.

In another aspect, a a bispecific antibody that binds to DR5 and FAP is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:7 and SEQ ID NO:8, and SEQ ID NO:15 and SEQ ID NO:16, respectively, including post-translational modifications of those sequences.

In a further aspect of the invention, a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment said bispecific antibody that binds to DR5 and FAP according to any of the above embodiments is a human antibody.

B. Exemplary antibodies that bind to DR5

In one aspect, the invention provides isolated antibodies and antibody fragments that bind to DR5. These death receptor agonistic antibodies have superior properties compared to known DR5 binders that can be incorporated into novel and advantageous bispecific antibodies targeting DR5 and a second antigen.

In one aspect, the invention provides an antibody that binds to death receptor 5 (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.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

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

(b) a heavy chain CDR2 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 one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:25;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:27;

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

(f) a light chain CDR3 of SEQ ID NO.:22.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:18;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

(a) a heavy chain CDR1 of SEQ ID NO.:17;

(b) a heavy chain CDR2 of SEQ ID NO.:25;

(c) a heavy chain CDR3 of SEQ ID NO.:19;

(d) a light chain CDR1 of SEQ ID NO.:20;

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

(f) a light chain CDR3 of SEQ ID NO.:22.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), comprising

(a) a heavy chain CDR1 of SEQ ID NO.:75;

(b) a heavy chain CDR2 of SEQ ID NO.:83;

(c) a heavy chain CDR3 of SEQ ID NO.:84;

(d) a light chain CDR1 of SEQ ID NO.:86;

(e) a light chain CDR2 of SEQ ID NO.:28;

(f) a light chain CDR3 of SEQ ID NO.:87.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), 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.:96;

(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.:99.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), 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.:104;

(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:105.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), 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.:108;

(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:109.

In one aspect, the invention provides an antibody that binds to death receptor 5 (DR5), 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.:98;

(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.:97.

In one embodiment, the antibody that binds to death receptor 5 (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.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises 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 one embodiment, the antibody that binds to death receptor 5 (DR5) comprisesa variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:24.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprisesa variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:24.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:29.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:30.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:31.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:32.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:26 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:30.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:23 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:31.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:82 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:85.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:100 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:101.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:102 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:103.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:106 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:107.

In one embodiment, the antibody that binds to death receptor 5 (DR5) comprises a variable heavy chain comprising an amino acid sequence of SEQ ID NO.:94 and a variable light chain comprising an amino acid sequence of SEQ ID NO.:95.

In another aspect, the antibody that binds to death receptor 5 (DR5) comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.:7.

In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but the antibody that binds to DR5 comprising that sequence retains the ability to bind to DR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.:7. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the antibody that binds to DR5 comprises the VH sequence in SEQ ID NO.:7, including post-translational modifications of that sequence.

In another aspect the antibody that binds to death receptor 5 (DR5) a light chain variable domain (VL) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO.:8.

In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an antibody that binds to DR5 comprising that sequence retains the ability to bind to DR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO.:8. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the antibody that binds to DR5 comprises the VL sequence in SEQ ID NO:8, including post-translational modifications of that sequence.

In another aspect, a bispecific antibody that binds to DR5 and a second antigen is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences in SEQ ID NO:7 and SEQ ID NO:8, respectively, including post-translational modifications of those sequences. In one embodiment a bispecific antibody is provided that binds to DR5 and a second antigen, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above and wherein the antibody has a format as outlined for the DR5-FAP bispecific antibodies in section C below. In another embodiment said bispecific antibody that binds to DR5 and a second antigen is provided which comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above, and which comprises one or more Fc domain modifications as outlined for the DR5-FAP bispecific antibodies in sections D and E below.

In a further aspect of the invention, an antibody that binds to DR5 according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment said antibody that binds to DR5 according to any of the above embodiments is a human antibody.

C. Exemplary formats of bispecific antibodies binding to DR5 and FAP

In one embodiment, a bispecific antibody that binds to DR5 and FAP comprises an antibody fragment, e.g., a Fv, Fab, Fab', scFv,xFab, scFab, diabody, or F(ab')$_2$ fragment. In another embodiment, the antibody comprises a full length antibody, e.g., an intact IgG1 antibody or other antibody class or isotype as defined herein.

The bispecific antibodies according to the invention are at least bivalent and can be trivalent or multivalent e.g. tetravalent or hexavalent.

The bispecific antibody of the invention comprise an Fc domain, at least one Fab fragment comprising an antigen binding site specific for DR5, and at least one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

In another embodiment, the bispecific antibody comprises an Fc domain, at least one Fab fragment comprising an antigen binding site specific for DR5, and at least one Fab fragment comprising an antigen binding site specific for FAP, wherein at least one of the Fab fragments is connected to the first or second subunit of the Fc domain via the light chain (VLCL) and at least one Fab fragment is connected to the first or second subunit of the Fc domain via the heavy chain (VHCH1).

In any of the embodiments, the Fab fragments may be fused to the Fc domain or to each other directly or through a peptide linker, comprising one or more amino acids, typically about 2-20 amino acids. Peptide linkers are known in the art and are described herein. Suitable, non-immunogenic peptide linkers include, for example, $(G_4S)_n$, $(SG_4)_n$, $(G_4S)_n$ or $G_4(SG_4)_n$ peptide linkers. "n" is generally a number between 1 and 10, typically between 2 and 4. A particularly suitable peptide linker for fusing the Fab light chains of the first and the second antigen binding moiety to each other is $(G_4S)_2$. An exemplary peptide linker suitable for connecting the Fab heavy chains of the first and the second antigen binding moiety is EPKSC(D)-$(G_4S)_2$ (SEQ ID NO.: 318). Additionally, linkers may comprise (a portion of) an immunoglobulin hinge region. Particularly where an antigen binding moiety is fused to the N-terminus of an Fc domain subunit, it may be fused via an immunoglobulin hinge region or a portion thereof, with or without an additional peptide linker.

Preferably said bispecific antibodies are tetravalent with two binding sites each targeting FAP and DR5, respectively (2+2 format). In another embodiment said bispecific antibodies are tetravalent with three binding sites for DR5 and one binding site for FAP (3+1 format). The 3+1 format can be achieved, for example, through fusing one Fab fragment targeting FAP and one

Fab fragment targeting DR5 to the C-terminus of the heavy chain of an IgG molecule that has two DR5 binding sites. This is outlined in more detail below.

In another preferred embodiment said bispecific antibodies are trivalent (2+1 format) with two binding sites each targeting DR5 and one binding site targeting FAP. The 2+1 format can be achieved, for example, through fusing a Fab fragment targeting FAP to the C-terminus of the heavy chain of an IgG molecule that has two DR5 binding sites., wherein the Fc part of the first antibody is modified according to the knobs-into hole strategy as outlined below.

In another preferred embodiment said bispecific antibodies are bivalent (1+1 format), i.e. monovalent for each DR5 and FAP. Bivalent antibodies of the invention have one binding site targeting DR5 and one binding site targeting FAP. The 1+1 format can be achieved, for example, by the Crossmab technology described in Schaefer et al. Proc Natl Acad Sci USA 2011; 108:11187-92 and as outlined below.

Exemplary formats of bispecific antibodies of the invention are given in Figure 25 and 28.

Provided therein are different bispecific antibody formats that are binding to DR5 and FAP comprising any of the sequences according to any of the above embodiments.

1. Bispecific DR5-FAP antibodies in a 2+2 Format

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP,

wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

Since the above bispecific antibody is bivalent both for FAP and DR5, with 2 binding sites each for FAP and DR5, this format is also referred to as “2+2” format. Exemplary structures of bispecific antibodies with a 2+2 format are depicted in Figure 28. Due to the exchange of either the variable regions or the constant regions, the Fab fragments above are also referred to as “cross-Fab fragment” or “xFab fragment” or “crossover Fab fragment”.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of both Fab fragments comprising an antigen binding site specific for FAP are exchanged.

In another embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP,

wherein either the variable regions or the constant regions of the heavy and light chain of both Fab fragments comprising an antigen binding site specific for DR5 are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP, wherein the variable regions of the heavy and light chain of both Fab fragments comprising an antigen binding site specific for FAP are exchanged.

In another embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP,

wherein the variable regions of the heavy and light chain of both Fab fragments comprising an antigen binding site specific for DR5 are exchanged.

Due to the exchange of the variable regions of the Fab heavy and light chain the crossover Fab fragments specific for FAP each comprise a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL).

These crossover Fab fragments are also referred to as CrossFab $_{(VLVH)}$ and each comprise a VLCH1 and a VHCL chain.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP,

wherein the constant regions of the heavy and light chain of both Fab fragments comprising an antigen binding site specific for FAP are exchanged.

In another embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and two Fab fragments comprising each an antigen binding site specific for FAP,

wherein the constant regions of the heavy and light chain of both Fab fragments comprising an antigen binding site specific for DR5 are exchanged.

Due to the exchange of the constant regions of the Fab heavy and light chain the crossover Fab fragments specific for FAP each comprise a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). These crossover Fab fragments are also referred to as CrossFab $_{(CLCH1}$ and comprise a VHCL and a VLCH1 chain.

In one embodiment said bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Fc domain to which two Fab fragments are fused to the N-terminus and two Fab fragments are fused to the C-terminus, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged. In one embodiment two Fab fragments are fused to the N-terminus of the Fc domain through an immunoglobulin hinge region. In one embodiment, the immunoglobulin hinge region is a human IgG1 hinge region. In one embodiment the two Fab fragments comprising an antigen binding site specific for DR5 and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more

particular embodiment the immunoglobulin is an IgG1 subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG4 subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.

In one embodiment two Fab fragments comprising the antigen binding site specific for FAP are connected to the Fc domain via a peptide linker. In one embodiment two Fab fragments comprising an antigen binding site specific for FAP are connected to the C-terminus of the first or second subunit of the Fc domain via a peptide linker. In one such embodiment said Fab fragments comprising an antigen binding site specific for FAP are connected to the C-terminus of the second subunit (CH3 chain) of the Fc domain via a peptide linker.

In one embodiment said bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 (i.e. two Fab fragments specific for DR5) and

b) two Fab fragments specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

In another embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 wherein either the variable regions or the constant regions of the heavy and light chain are exchanged and

b) two Fab fragments specific for FAP.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule.

In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain of said IgG molecule. In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain of said IgG molecule.

In one embodiment said two Fab fragments are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule.

In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain of said IgG molecule. In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain of said IgG molecule. In one embodiment said two Fab fragments are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule.

In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain of said IgG molecule. In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain of said IgG molecule. In one embodiment said two Fab fragments are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In a further preferred embodiment, the two Fab fragments specific for FAP are fused to the IgG molecule by a peptide linker, preferably a peptide linker having a length of about 10 – 30 amino acids. Preferably said peptide linker is a (G4S)2 or (G4S)4 linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain of said IgG molecule by a peptide linker. In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain of said IgG molecule by a peptide linker.

In one embodiment said two Fab fragments are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) two Fab fragments specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment said two Fab fragments are fused to the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain of said IgG molecule by a peptide linker.

In one embodiment said two Fab fragments are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule by a peptide linker.

In one embodiment, the bispecific antibody comprises an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP, wherein at least one of the Fab fragments is connected to the first or second subunit of the Fc domain via the light chain (VLCL) and at least one Fab fragment is connected to the first or second subunit of the Fc domain via the heavy chain (VHCH1).

In one embodiment, the bispecific antibody comprises an Fc domain, two Fab fragments comprising the antigen binding site specific for DR5, and two Fab fragments comprising the antigen binding site specific for FAP, wherein at least one of the Fab fragments is fused to the first or second subunit of the Fc domain via the light chain (VLCL) and at least one Fab fragment is connected to the first or second subunit of the Fc domain via the heavy chain (VHCH1).

In one embodiment, the bispecific antibody comprises

a) an Fc domain,

b) two Fab fragments comprising an antigen binding site specific for DR5,
wherein said Fab fragments are connected at the C-terminus of the constant light chain (CL) to the first or second subunit of the Fc domain,

c) two Fab fragments comprising the antigen binding site specific for FAP, wherein the two Fab fragments are connected at the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain.

In one embodiment, the bispecific antibody comprises

a) an Fc domain,

b) two Fab fragments comprising an antigen binding site specific for DR5,

wherein said Fab fragments are connected at the C-terminus of the constant light chain (CL) to the N-terminus of the first subunit of the Fc domain,

c) two Fab fragments comprising the antigen binding site specific for FAP, wherein the two Fab fragments are connected at the C-terminus of the constant heavy chain (CH1) to the second subunit (CH3) of the Fc domain.

In one embodiment, said two Fab fragments comprising an antigen binding site specific for DR5 are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG1 hinge region. In one embodiment the two Fab fragments comprising an antigen binding site specific for DR5 and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG1 subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG4 subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.
In one embodiment two Fab fragments comprising the antigen binding site specific for FAP are connected to the Fc domain via a peptide linker.

In one embodiment, the bispecific antibody comprises

a) an Fc domain,

b) two Fab fragments comprising an antigen binding site specific for DR5,
wherein said Fab fragments are connected at the C-terminus of the constant heavy chain (CH1) to the first or second subunit of the Fc domain,

c) two Fab fragments comprising the antigen binding site specific for FAP, wherein the two Fab fragments are connected at the N-terminus of the constant light chain (CL) to the first or second subunit of the Fc domain.

In one embodiment, the bispecific antibody comprises

a) an Fc domain,

b) two Fab fragments comprising an antigen binding site specific for DR5,
wherein said Fab fragments are connected at the C-terminus of the constant heavy chain (CH1) to the N- terminus of the first subunit of the Fc domain,

c) two Fab fragments comprising the antigen binding site specific for FAP, wherein the two Fab fragments are connected at the N-terminus of the constant light chain (CL) to the N-terminus of the second subunit of the Fc domain.

In one embodiment, said two Fab fragments comprising an antigen binding site specific for DR5 are each fused to the Fc domain through an immunoglobulin hinge region. In a specific embodiment, the immunoglobulin hinge region is a human IgG1 hinge region. In one embodiment the two Fab fragments comprising an antigen binding site specific for DR5 and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG1 subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG4 subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.

Exemplary antibodies with a 2+2 format

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising

a heavy chain CDR1 of SEQ ID NO.:1;
a heavy chain CDR2 of SEQ ID NO.:2;
a heavy chain CDR3 of SEQ ID NO.:3;
a light chain CDR1 of SEQ ID NO.:4;
a light chain CDR2 of SEQ ID NO.:5;
a light chain CDR3 of SEQ ID NO.:6; and

b) two Fab fragments specific for FAP, comprising

a heavy chain CDR1 of SEQ ID NO.:9;
a heavy chain CDR2 of SEQ ID NO.:10;
a heavy chain CDR3 of SEQ ID NO.:11;
a light chain CDR1 of SEQ ID NO.:12;
a light chain CDR2 of SEQ ID NO.:13;
a light chain CDR3 of SEQ ID NO.:14;

wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising

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

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

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

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

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

a light chain CDR3 of SEQ ID NO.:6; and

b) two Fab fragments specific for FAP, comprising

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

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

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

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

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

a light chain CDR3 of SEQ ID NO.:14;

wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8; and

b) two Fab fragments specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16; wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8; and

b) two Fab fragments specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16; wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In another embodiment said bispecific antibody of the invention comprises a modification in the Fc part of the IgG molecule, as outlined below.

In one embodiment a bispecific antibody with a 2+2 format as described above is provided comprising two VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chains of SEQ ID NO.:131, two VL (DR5)-kappa light chains of SEQ ID NO.:132 and two VLCH1 (FAP) chains of SEQ ID NO.:124.

In one embodiment a bispecific antibody with a 2+2 format as described above is provided comprising two VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chains of SEQ ID NO.:133, two VL (DR5)-kappa light chains of SEQ ID NO.:132 and two VLCH1 (FAP) chains of SEQ ID NO.:124.

In one embodiment a bispecific antibody with a 2+2 format as described above is provided comprising two VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chains of SEQ ID NO.:134, two VL (DR5)-kappa light chains of SEQ ID NO.:132 and two VLCH1 (FAP) chains of SEQ ID NO.:124.

In one embodiment a bispecific antibody with a 2+2 format as described above is provided comprising two VL $_{(DR5)}$-CH1- Fc part-VH$_{(FAP)}$-CH1 chains of SEQ ID NO. 135, two

$VH_{(DR5)}$ –CL chains of SEQ ID NO.:136 and two $VL_{(FAP)}$ –kappa light chains of SEQ ID NO.:137.

In one embodiment a bispecific antibody with a 2+2 format as described above is provided, comprising two $VH_{(DR5)}$ CL- Fc- peptide linker-$VH_{(FAP)}$ -CH1 chains of SEQ ID NO.:138, two $VL_{(DR5)}$ –CH1 chains of SEQ ID NO.:139 and two $VL_{(FAP)}$ –kappa light chains of SEQ ID NO.:137.

2. Bispecific DR5-FAP antibodies in a 2+1 Format

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising an antigen binding site specific for DR5,

and one Fab fragment comprising the antigen binding site specific for FAP,

wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

Since the above bispecific antibodies are trivalent with one binding site for FAP and two binding sites for DR5, this format is also referred to as “2+1” format. Hence the bispecific antibodies provided in this section are bivalent for DR5 and monovalent for FAP.

An exemplary structure of a bispecific antibodies with a 2+1 format are depicted in Figure 25 a) and b). Due to the exchange of either the variable regions or the constant regions, the Fab fragments above are also referred to as “cross-Fab fragment” or “xFab fragment” or “crossover Fab fragment”.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of the Fab fragments comprising an antigen binding site specific for FAP are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein the variable regions of the heavy and light chain of the Fab fragment comprising an antigen binding site specific for FAP are exchanged.

Due to the exchange of the variable regions of the Fab heavy and light chain the crossover Fab fragment specific for FAP each comprise a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). These crossover Fab fragment is also referred to as CrossFab $_{(VLVH)}$ and comprises a VLCH1 and a VHCL chain.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

two Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein the constant regions of the heavy and light chain of both Fab fragments comprising the antigen binding site specific for FAP are exchanged.

Due to the exchange of the constant regions of the Fab heavy and light chain the crossover Fab fragment specific for FAP each comprise a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). These crossover Fab fragments is also referred to as CrossFab $_{(CLCH1)}$ and comprises a VHCL and a VLCH1 chain.

In one embodiment said bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Fc domain to which two Fab fragments are fused to the N-terminus.

In one embodiment two Fab fragments are fused to the N-terminus of the Fc domain through an immunoglobulin hinge region. In one embodiment, the immunoglobulin hinge region is a human IgG1 hinge region. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG1 subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG4 subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human

immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.

Bispecific DR5-FAP antibodies in a 2+1 Format with FAP binder fused to C-terminus

In one embodiment the two Fab fragments comprising an antigen binding site specific for DR5 and the Fc domain are part of an immunoglobulin molecule. In one embodiment one Fab fragment comprising the antigen binding site specific for FAP is fused to the C-terminus of the first or second subunit of the Fc domain via a peptide linker. In one such embodiment said Fab fragment comprising an antigen binding site specific for FAP is fused to the C-terminus of the second subunit (CH3 chain) of the Fc domain via a peptide linker. An exemplary structure of a bispecific DR5-FAP antibody in a 2+1 Format with the FAP binder fused to C-terminus is depicted in Figure 25 a).

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) one Fab fragment specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) one Fab fragment specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) one Fab fragment specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged, wherein the Fab fragment is fused to the constant heavy chain of said IgG molecule.

In one embodiment said Fab fragment specific for FAP of b) is fused to the C-terminus of the first or second subunit of the Fc domain of said IgG molecule. In one embodiment said Fab fragment of b) is fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) one Fab fragment specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the Fab fragment is fused to the constant heavy chain of said IgG molecule.

In one embodiment said Fab fragment specific for FAP of b) is fused to the C-terminus of the first or second subunit of the Fc domain of said IgG molecule. In one embodiment said Fab fragment of b) is fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5 and

b) one Fab fragment specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the Fab fragment is fused to the constant heavy chain of said IgG molecule.

In one embodiment said Fab fragment specific for FAP of b) is fused to the C-terminus of the first or second subunit of the Fc domain of said IgG molecule. In one embodiment said Fab

fragment of b) is fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

Bispecific DR5-FAP antibodies in a 2+1 Format with FAP binder fused to the N-terminus

In another embodiment one Fab fragment comprising an antigen binding site specific for DR5, one Fab fragment comprising an antigen binding site specific for FAP and the Fc domain are part of an immunoglobulin molecule. In one embodiment another Fab fragment comprising an antigen binding site specific for DR5 is fused to the N-terminus of the Fab fragment comprising an antigen binding site specific for DR5 of the IgG molecule via a peptide linker. An exemplary structure of a bispecific DR5-FAP antibody in a 2+1 Format with the FAP binder fused to N-terminus is depicted in Figure 25 b).

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with one binding site (Fab fragment) specific for DR5 and one binding site (Fab fragment) specific for FAP, wherein the Fab fragment specific for FAP is a Crossfab fragment (i.e. either the variable regions or the constant regions of the heavy and light chain are exchanged).

b) one Fab fragment specific for DR5.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the Fab fragment specific for FAP is a CrossFab $_{(VLVH)}$ fragment (i.e. the variable regions of the heavy and light chain are exchanged).

b) one Fab fragment specific for DR5.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the Fab fragment specific for FAP is a CrossFab $_{(CLCH1}$ fragment (i.e. the constant regions of the heavy and light chain are exchanged).

b) one Fab fragment specific for DR5.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the Fab fragment specific for FAP is a Crossfab fragment (i.e. either the variable regions or the constant regions of the heavy and light chain are exchanged).

b) one Fab fragment specific for DR5, wherein said Fab fragment is fused to the N-terminus of the variable heavy or light chain of the IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with one binding site (Fab fragment) specific for DR5 and one binding site specific for FAP, wherein the Fab fragment specific for FAP is a CrossFab $_{(VLVH)}$ fragment (i.e. the variable regions of the heavy and light chain are exchanged).

b) one Fab fragment specific for DR5, wherein said Fab fragment is fused to the N-terminus of the variable heavy or light chain of the IgG molecule.

In one embodiment, the Fab fragment specific for DR5 of b) is fused to the N-terminus of the variable heavy or light chain of the Fab fragment specific for DR5 of a).

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the Fab fragment specific for FAP is a CrossFab $_{(CLCH1}$ fragment (i.e. the constant regions of the heavy and light chain are exchanged).

b) one Fab fragment specific for DR5, wherein said Fab fragment is fused to the N-terminus of the variable heavy or light chain of the IgG molecule

In one embodiment, the Fab fragment specific for DR5 of b) is fused to the N-terminus of the variable heavy or light chain of the Fab fragment specific for DR5 of a).

In a further preferred embodiment, the Fab fragment specific for DR5 is fused to the IgG molecule by a peptide linker, preferably a peptide linker having a length of about 10 – 30 amino acids. Preferably said peptide linker is a (G4S)2 or (G4S)4 linker.

In another embodiment said bispecific antibody of the invention comprises a modification in the Fc part of the IgG molecule, as outlined below.

3. Bispecific DR5-FAP antibodies in a 3+1 Format

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

three Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP,

wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

Since the above bispecific antibody is tetravalent with one binding site for FAP and three binding sites for DR5, this format is also referred to as "3+1" format. Hence the bispecific molecules described in this section are trivalent for DR5 and monovalent for FAP.

An exemplary structure of a bispecific antibody with a 3 +1 format is depicted in Figure 25 c). Due to the exchange of either the variable regions or the constant regions, the Fab fragments above are also referred to as "cross-Fab fragment" or "xFab fragment" or "crossover Fab fragment".

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

three Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of the Fab fragments comprising an antigen binding site specific for FAP are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

three Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein the variable regions of the heavy and light chain of the Fab fragment comprising an antigen binding site specific for FAP are exchanged.

Due to the exchange of the variable regions of the Fab heavy and light chain the crossover Fab fragment specific for FAP comprises a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1), and a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL). This crossover Fab fragments is also referred to as CrossFab $_{(VLVH)}$ and comprises a VLCH1 and a VHCL chain.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

three Fab fragments comprising each an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein the constant regions of the heavy and light chain of the Fab fragment comprising the antigen binding site specific for FAP are exchanged.

Due to the exchange of the constant regions of the Fab heavy and light chain the crossover Fab fragment specific for FAP each comprise a peptide chain composed of the heavy chain variable region (VH) and the light chain constant region (CL), and a peptide chain composed of the light chain variable region (VL) and the heavy chain constant region (CH1). This crossover Fab fragment is also referred to as CrossFab $_{(CLCH1)}$ and comprise a VHCL and a VLCH1 chain.

In one embodiment said bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Fc domain to which two Fab fragments are fused to the N terminus and two Fab fragments are fused to the C-terminus, wherein either the variable regions or the constant regions of the heavy and light chain of the one Fab fragment specific fro FAP are exchanged.

In one embodiment two Fab fragments are fused to the N-terminus of the Fc domain through an immunoglobulin hinge region. In one embodiment, the immunoglobulin hinge region is a human IgG1 hinge region. In one embodiment the two Fab fragments comprising an antigen binding site specific for DR5 and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG1 subclass immunoglobulin. In another

embodiment the immunoglobulin is an IgG4 subclass immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

c) one Fab fragment specific for DR5.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged.

c) one Fab fragment specific for DR5.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged.

c) one Fab fragment specific for DR5.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

c) one Fab fragment specific for DR5,

wherein the Fab fragments of b) and c) are fused to the C-terminus of the first or second subunit of the Fc domain of said IgG molecule.

In one embodiment said two Fab fragments of b) and c) are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged.

c) one Fab fragment specific for DR5,

wherein the Fab fragments of b) and c) are fused to the first or second subunit of the Fc domain of said IgG molecule.

In one embodiment said two Fab fragments of b) and c) are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites (Fab fragments) specific for DR5 and

b) one Fab fragment specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged.

c) one Fab fragment specific for DR5,

wherein the Fab fragments of b) and c) are fused to the first or second subunit of the Fc domain of said IgG molecule.

In one embodiment said two Fab fragments of b) and c) are fused to the C-terminus of the second subunit (CH3) of the Fc domain of said IgG molecule.

In a further preferred embodiment, the two Fab fragments of b) and c) of any of the embodiments described in this section are fused to the IgG molecule by a peptide linker, preferably a peptide linker having a length of about 10 – 30 amino acids. Preferably said peptide linker is a (G4S)2 or (G4S)4 linker.

In another embodiment said bispecific antibody of the invention comprises a modification in the Fc part of the IgG molecule, as outlined below.

4. Bispecific DR5-FAP antibodies in a 1+1 Format

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

one Fab fragment comprising an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP,

wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged.

Since the above bispecific antibody is bivalent with one binding site for FAP and one binding site for DR5, this format is also referred to as "1+1" format. Hence the bispecific antibodies described in this section are monovalent for DR5 and monovalent for FAP. An exemplary structure of a bispecific antibody with a 1 +1 format is depicted in Figure 25 d). Due to the exchange of either the variable regions or the constant regions, the Fab fragment above is also referred to as "cross-Fab fragment" or "xFab fragment" or "crossover Fab fragment". The IgG molecule in a 1+1 format is also referred to as Crossmab format (see Schaefer et al. Proc Natl Acad Sci USA 2011; 108:11187-92).

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

one Fab fragment comprising an antigen binding site specific for DR5, wherein either the variable regions or the constant regions of the heavy and light chain of the Fab fragment comprising an antigen binding site specific for DR5 are exchanged.

and one Fab fragment comprising an antigen binding site specific for FAP.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

one Fab fragment comprising an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of the Fab fragment comprising an antigen binding site specific for FAP are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

one Fab fragment comprising an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein the variable regions of the heavy and light chain of the Fab fragment comprising an antigen binding site specific for FAP are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

an Fc domain,

one Fab fragments comprising an antigen binding site specific for DR5,

and one Fab fragment comprising an antigen binding site specific for FAP, wherein the constant regions of the heavy and light chain of the Fab fragment comprising the antigen binding site specific for FAP are exchanged.

In one embodiment said bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Fc domain to which two Fab fragments are fused to the N-terminus, wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged. In one embodiment the two Fab fragments are fused to the N-terminus of the Fc domain through an immunoglobulin hinge region. In one embodiment, the immunoglobulin hinge region is a human IgG1 hinge region. In one embodiment the Fab fragment comprising an antigen binding site specific for DR5, the Fab fragment comprising an antigen binding site specific for FAP and the Fc domain are part of an immunoglobulin molecule. In a particular embodiment the immunoglobulin molecule is an IgG class immunoglobulin. In an even more particular embodiment the immunoglobulin is an IgG1 subclass immunoglobulin. In another embodiment the immunoglobulin is an IgG4 subclass

immunoglobulin. In a further particular embodiment the immunoglobulin is a human immunoglobulin. In other embodiments the immunoglobulin is a chimeric immunoglobulin or a humanized immunoglobulin.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain of one arm (Fab fragment) of the IgG molecule are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the variable regions of the heavy and light chain of one arm (Fab fragment) of the IgG molecule are exchanged. This antibody format is also referred to as CrossMab($_{VHVL}$).

In one embodiment the variable regions of the heavy and light chain of the one arm (Fab fragment) of the IgG molecule which comprises the binding site specific for FAP are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the constant regions of the heavy and light chain of one arm (Fab fragment) of the IgG molecule are exchanged. This antibody format is also referred to as CrossMab$_{(CH1CL)}$.

In one embodiment the constant regions of the heavy and light chain of the one arm (Fab fragment) of the IgG molecule which comprises the binding site specific for FAP are exchanged.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Immunoglobulin G (IgG) molecule with one binding site specific for DR5 and one binding site specific for FAP, wherein the complete VH-CH1 and VL-CL domains of one arm (Fab fragment) of the IgG molecule are exchanged. This means that at least one of the Fab fragments is fused to the N-terminus of the Fc domain via the light chain (VLCL). In one embodiment the other Fab fragment is fused to the the N-terminus of the Fc domain via the heavy chain (VHCH1).

This antibody format is also referred to as $CrossMab_{Fab}$. In one embodiment both Fab fragments are are fused to the N-terminus of the Fc domain through an immunoglobulin hinge region.

D. Fc domain modifications reducing Fc receptor binding and/or effector function

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Immunoglobulin G (IgG) molecule wherein the Fc part is modified. The modified Fc part has a reduced binding affinity for the Fcγ receptors compared to a wildtype Fc part.

The Fc domain of the bispecific antibodies of the invention consists of a pair of polypeptide chains comprising heavy chain domains of an immunoglobulin molecule. For example, the Fc domain of an immunoglobulin G (IgG) molecule is a dimer, each subunit of which comprises the CH2 and CH3 IgG heavy chain constant domains. The two subunits of the Fc domain are capable of stable association with each other.

In one embodiment according the invention the Fc domain of the bispecific antibodies of the invention is an IgG Fc domain. In a particular embodiment the Fc domain is an IgG_1 Fc domain. In another embodiment the Fc domain is an IgG_4 Fc domain. In a more specific embodiment, the Fc domain is an IgG_4 Fc domain comprising an amino acid substitution at position S228 (Kabat numbering), particularly the amino acid substitution S228P. In a more specific embodiment, the Fc domain is an IgG_4 Fc domain comprising amino acid substitutions L235E and S228P and P329G. This amino acid substitution reduces in vivo Fab arm exchange of IgG_4 antibodies (see Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). In a further particular embodiment the Fc domain is human. An exemplary sequence of a human IgG_1 Fc region is given in SEQ ID NO.:151.

The Fc domain confers favorable pharmacokinetic properties to the bispecific antibodies of the invention, including a long serum half-life which contributes to good accumulation in the target tissue and a favorable tissue-blood distribution ratio. At the same time it may, however, lead to undesirable targeting of the bispecific antibodies of the invention to cells expressing Fc receptors rather than to the preferred antigen-bearing cells. Accordingly, in particular embodiments the Fc domain of the the bispecific antibodies of the invention exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG_1 Fc domain. In one such embodiment the Fc domain (or the bispecific antibodies of the invention comprising said Fc domain) exhibits less than 50%, preferably less than 20%, more preferably less than 10% and

most preferably less than 5% of the binding affinity to an Fc receptor, as compared to a native IgG_1 Fc domain (or a bispecific antibodies of the invention comprising a native IgG_1 Fc domain), and/or less than 50%, preferably less than 20%, more preferably less than 10% and most preferably less than 5% of the effector function, as compared to a native IgG_1 Fc domain domain (or a bispecific antibodies of the invention comprising a native IgG_1 Fc domain). In one embodiment, the Fc domain (or the bispecific antibodies of the invention comprising said Fc domain) does not substantially bind to an Fc receptor and/or induce effector function. In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one embodiment the Fc receptor is an inhibitory Fc receptor. In a specific embodiment the Fc receptor is an inhibitory human Fcγ receptor, more specifically human FcgRIIB. In one embodiment the effector function is one or more of CDC, ADCC, ADCP, and cytokine secretion. In a particular embodiment the effector function is ADCC. In one embodiment the Fc domain domain exhibits substantially similar binding affinity to neonatal Fc receptor (FcRn), as compared to a native IgG_1 Fc domain domain. Substantially similar binding to FcRn is achieved when the Fc domain (or the bispecific antibodies of the invention comprising said Fc domain) exhibits greater than about 70%, particularly greater than about 80%, more particularly greater than about 90% of the binding affinity of a native IgG_1 Fc domain (or the bispecific antibodies of the invention comprising a native IgG_1 Fc domain) to FcRn.

In certain embodiments the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In particular embodiments, the Fc domain of the bispecific antibodies of the invention comprises one or more amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function. Typically, the same one or more amino acid mutation is present in each of the two subunits of the Fc domain. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor. In one embodiment the amino acid mutation reduces the binding affinity of the Fc domain to an Fc receptor by at least 2-fold, at least 5-fold, or at least 10-fold. In embodiments where there is more than one amino acid mutation that reduces the binding affinity of the Fc domain to the Fc receptor, the combination of these amino acid mutations may reduce the binding affinity of the Fc domain to an Fc receptor by at least 10-fold, at least 20-fold, or even at least 50-fold. In one embodiment the bispecific

antibodies of the invention comprising an engineered Fc domain exhibits less than 20%, particularly less than 10%, more particularly less than 5% of the binding affinity to an Fc receptor as compared to a bispecific antibodies of the invention comprising a non-engineered Fc domain. In a particular embodiment the Fc receptor is an Fcγ receptor. In some embodiments the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an inhibitory Fc receptor. In a specific embodiment the Fc receptor is an inhibitory human Fcγ receptor, more specifically human FcgRIIB. In some embodiments the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. Preferably, binding to each of these receptors is reduced. In some embodiments binding affinity to a complement component, specifically binding affinity to C1q, is also reduced. In one embodiment binding affinity to neonatal Fc receptor (FcRn) is not reduced. Substantially similar binding to FcRn, i.e. preservation of the binding affinity of the Fc domain to said receptor, is achieved when the Fc domain (or the bispecific antibodies of the invention comprising said Fc domain) exhibits greater than about 70% of the binding affinity of a non-engineered form of the Fc domain (or the bispecific antibodies of the invention comprising said non-engineered form of the Fc domain) to FcRn. The Fc domain, or the bispecific antibodies of the invention of the invention comprising said Fc domain, may exhibit greater than about 80% and even greater than about 90% of such affinity. In certain embodiments the Fc domain of the bispecific antibodies of the invention is engineered to have reduced effector function, as compared to a non-engineered Fc domain. The reduced effector function can include, but is not limited to, one or more of the following: reduced complement dependent cytotoxicity (CDC), reduced antibody-dependent cell-mediated cytotoxicity (ADCC), reduced antibody-dependent cellular phagocytosis (ADCP), reduced cytokine secretion, reduced immune complex-mediated antigen uptake by antigen-presenting cells, reduced binding to NK cells, reduced binding to macrophages, reduced binding to monocytes, reduced binding to polymorphonuclear cells, reduced direct signaling inducing apoptosis, reduced dendritic cell maturation, or reduced T cell priming. In one embodiment the reduced effector function is one or more of reduced CDC, reduced ADCC, reduced ADCP, and reduced cytokine secretion. In a particular embodiment the reduced effector function is reduced ADCC. In one embodiment the reduced ADCC is less than 20% of the ADCC induced by a non-engineered Fc domain (or a bispecific antibody of the invention comprising a non-engineered Fc domain).

In one embodiment the amino acid mutation that reduces the binding affinity of the Fc domain to an Fc receptor and/or effector function is an amino acid substitution. In one embodiment the Fc domain comprises an amino acid substitution at a position of E233, L234, L235, N297, P331 and P329. In a more specific embodiment the Fc domain comprises an amino acid substitution at a position of L234, L235 and P329. In some embodiments the Fc domain comprises the amino acid substitutions L234A and L235A. In one such embodiment, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain. In one embodiment the Fc domain comprises an amino acid substitution at position P329. In a more specific embodiment the amino acid substitution is P329A or P329G, particularly P329G. In one embodiment the Fc domain comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331. In a more specific embodiment the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments the Fc domain comprises amino acid substitutions at positions P329, L234 and L235. In more particular embodiments the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA"). In one such embodiment, the Fc domain is an IgG_1 Fc domain, particularly a human IgG_1 Fc domain. The "P329G LALA" combination of amino acid substitutions almost completely abolishes Fcγ receptor binding of a human IgG_1 Fc domain, as described in PCT patent application no. PCT/EP2012/055393, incorporated herein by reference in its entirety. PCT/EP2012/055393 also describes methods of preparing such mutant Fc domains and methods for determining its properties such as Fc receptor binding or effector functions.

IgG_4 antibodies exhibit reduced binding affinity to Fc receptors and reduced effector functions as compared to IgG_1 antibodies. Hence, in some embodiments the Fc domain of the bispecific antibodies of the invention is an IgG_4 Fc domain, particularly a human IgG_4 Fc domain. In one embodiment the IgG_4 Fc domain comprises amino acid substitutions at position S228, specifically the amino acid substitution S228P. To further reduce its binding affinity to an Fc receptor and/or its effector function, in one embodiment the IgG_4 Fc domain comprises an amino acid substitution at position L235, specifically the amino acid substitution L235E. In another embodiment, the IgG_4 Fc domain comprises an amino acid substitution at position P329, specifically the amino acid substitution P329G. In a particular embodiment, the IgG_4 Fc domain comprises amino acid substitutions at positions S228, L235 and P329, specifically amino acid substitutions S228P, L235E and P329G. Such IgG_4 Fc domain mutants and their Fcγ receptor

binding properties are described in PCT patent application no. PCT/EP2012/055393, incorporated herein by reference in its entirety.

In a particular embodiment the Fc domain exhibiting reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG_1 Fc domain, is a human IgG_1 Fc domain comprising the amino acid substitutions L234A, L235A and optionally P329G, or a human IgG_4 Fc domain comprising the amino acid substitutions S228P, L235E and optionally P329G.

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

In addition to the Fc domains described hereinabove and in PCT patent application no. PCT/EP2012/055393, Fc domains with reduced Fc receptor binding and/or effector function also include those with substitution of one or more of Fc domain residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

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

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

Effector function of an Fc domain, or bispecific antibodies of the invention comprising an Fc domain, can be measured by methods known in the art. A suitable assay for measuring ADCC is described herein. Other examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362; Hellstrom et al. Proc Natl Acad Sci USA 83, 7059-7063 (1986) and Hellstrom et al., Proc Natl Acad Sci USA 82, 1499-1502 (1985); U.S.

Patent No. 5,821,337; Bruggemann et al., J Exp Med 166, 1351-1361 (1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA); and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI)). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g. in a animal model such as that disclosed in Clynes et al., Proc Natl Acad Sci USA 95, 652-656 (1998).

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

The following section describes preferred embodiments of the bispecific antibodies of the invention comprising Fc domain modifications reducing Fc receptor binding and/or effector function.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, wherein the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native IgG1 Fc domain and

b) two Fab fragments specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

b) two Fab fragments specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, wherein said one or more amino acid substitution is at one or more position of L234, L235, and P329

b) two Fab fragments specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, 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.

b) two Fab fragments specific for FAP, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, 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.

b) two Fab fragments specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged.

In one preferred embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, 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.

b) two Fab fragments specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged.

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In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, 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 and

b) two Fab fragments specific for FAP, wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, 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 and

b) two Fab fragments specific for FAP, wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising

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

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

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

a light chain CDR1 of SEQ ID NO.:4;
a light chain CDR2 of SEQ ID NO.:5;
a light chain CDR3 of SEQ ID NO.:6;

wherein each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to activating and inhibitory Fc receptors and/or effector function wherein said amino acid substitutions are L234A, L235A and P329G and

b) two Fab fragments specific for FAP, comprising
a heavy chain CDR1 of SEQ ID NO.:9;
a heavy chain CDR2 of SEQ ID NO.:10;
a heavy chain CDR3 of SEQ ID NO.:11;
a light chain CDR1 of SEQ ID NO.:12;
a light chain CDR2 of SEQ ID NO.:13;
a light chain CDR3 of SEQ ID NO.:14;

wherein either the variable regions or the constant regions of the heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising
a heavy chain CDR1 consisting of SEQ ID NO.:1;
a heavy chain CDR2 of SEQ ID NO.:2;
a heavy chain CDR3 of SEQ ID NO.:3;
a light chain CDR1 of SEQ ID NO.:4;
a light chain CDR2 of SEQ ID NO.:5;
a light chain CDR3 of SEQ ID NO.:6;

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 and

b) two Fab fragments specific for FAP, comprising
a heavy chain CDR1 of SEQ ID NO.:9;
a heavy chain CDR2 of SEQ ID NO.:10;
a heavy chain CDR3 of SEQ ID NO.:11;
a light chain CDR1 of SEQ ID NO.:12;
a light chain CDR2 of SEQ ID NO.:13;
a light chain CDR3 of SEQ ID NO.:14;

wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising
a heavy chain CDR1 consisting of SEQ ID NO.:1;
a heavy chain CDR2 of SEQ ID NO.:2;
a heavy chain CDR3 of SEQ ID NO.:3;
a light chain CDR1 of SEQ ID NO.:4;
a light chain CDR2 of SEQ ID NO.:5;
a light chain CDR3 of SEQ ID NO.:6;

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 and

b) two Fab fragments specific for FAP, comprising
a heavy chain CDR1 of SEQ ID NO.:9;
a heavy chain CDR2 of SEQ ID NO.:10;
a heavy chain CDR3 of SEQ ID NO.:11;

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

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

a light chain CDR3 of SEQ ID NO.:14;

wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8;

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 and

b) two Fab fragments specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16, wherein either the variable regions or the constant regions of the heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8;

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 and

b) two Fab fragments specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region

comprising an amino acid sequence of SEQ ID NO.:16wherein the constant regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises

a) an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, comprising a variable heavy chain of SEQ ID NO.:7 and a variable light chain of SEQ ID NO.:8;

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 and

b) two Fab fragments specific for FAP, comprising a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16, wherein the variable regions of the Fab heavy and light chain are exchanged, wherein the two Fab fragments are fused to the constant heavy chain of said IgG molecule by a peptide linker.

E. Fc domain modifications promoting heterodimerization

The bispecific DR5-FAP antibodies of the invention comprise different antigen binding moieties, fused to one or the other of the two subunits of the Fc domain, thus the two subunits of the Fc domain are typically comprised in two non-identical polypeptide chains. Recombinant co-expression of these polypeptides and subsequent dimerization leads to several possible combinations of the two polypeptides. To improve the yield and purity of the bispecific antibodies of the invention in recombinant production, it will thus be advantageous to introduce in the Fc domain of the bispecific antibodies of the invention a modification promoting the association of the desired polypeptides.

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

In a specific embodiment said modification is a so-called "knob-into-hole" modification, comprising a "knob" modification in one of the two subunits of the Fc domain and a "hole" modification in the other one of the two subunits of the Fc domain.

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

Accordingly, in a particular embodiment, in the CH3 domain of the first subunit of the Fc domain of the bispecific antibodies of the invention an amino acid residue is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and in the CH3 domain of the second subunit of the Fc domain an amino acid residue is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable.

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

In a specific embodiment, in the CH3 domain of the first subunit of the Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the CH3 domain of the second subunit of the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V). In one embodiment, in the second subunit of the Fc domain additionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A).

In yet a further embodiment, in the first subunit of the Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C), and in the second subunit of the Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C). Introduction of these two cysteine residues results in formation of a disulfide bridge

between the two subunits of the Fc domain, further stabilizing the dimer (Carter, J Immunol Methods 248, 7-15 (2001)).

In an alternative embodiment a modification promoting association of the first and the second subunit of the Fc domain comprises a modification mediating electrostatic steering effects, e.g. as described in PCT publication WO 2009/089004. Generally, this method involves replacement of one or more amino acid residues at the interface of the two Fc domain subunits by charged amino acid residues so that homodimer formation becomes electrostatically unfavorable but heterodimerization electrostatically favorable.

In one embodiment a bispecific antibody that binds to DR5 and FAP according to any of the above embodiments comprises an Immunoglobulin G (IgG) molecule with two binding sites specific for DR5, 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 IgG molecule.

In a further preferred embodiment, the first dimerization module comprises knobs and the second dimerization module comprises holes according to the knobs into holes strategy (see Carter P.; Ridgway J.B.B.; Presta L.G.: Immunotechnology, Volume 2, Number 1, February 1996 , pp. 73-73(1)).

F. Nucleic Acid sequences, vectors and methods of

The invention further provides isolated polynucleotides encoding a bispecific antibody or an antibody binding to DR5 as described herein or a fragment thereof. The polynucleotides encoding bispecific antibodies or the antibodies binding to DR5 of the invention may be expressed as a single polynucleotide that encodes the entire bispecific antigen binding molecule or the entire antibody binding to DR5 or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional bispecific antibody or an antibody binding to DR5. For example, the light chain portion of a Fab fragment may be encoded by a separate polynucleotide from the portion of the bispecific antibody or the antibody binding to DR5 comprising the heavy chain portion of the Fab fragment, an Fc domain subunit and optionally (part of) another Fab fragment. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the Fab fragment. In another example, the portion of the bispecific antibody or the antibody binding to DR5 provided therein comprising one of the two Fc domain subunits and optionally (part of) one or more Fab fragments could be encoded by a separate polynucleotide from the portion of the bispecific

antibody or the antibody binding to DR5 provided therein comprising the the other of the two Fc domain subunits and optionally (part of) a Fab fragment. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In one embodiment, the present invention is directed to an isolated polynucleotide encoding a bispecific antibody of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable heavy chain sequence as shown in SEQ ID NOs 167, 175, 183, 191, 199, 207 and 209.

In one embodiment, the present invention is directed to an isolated polynucleotide encoding a bispecific antibody of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable light chain sequence as shown in SEQ ID NOs 171, 179, 187, 195, 203, 208 and 210.

In another embodiment, the present invention is directed to an isolated polynucleotide encoding a bispecific antibody or fragment thereof, wherein the polynucleotide comprises a sequence that encodes a polypeptide sequence as shown in SEQ ID NOs 222, 224, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 258, 259, 260, 261, 264 and 265.

In one embodiment, the present invention is directed to an isolated polynucleotide encoding an antibody binding to DR5 of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable heavy chain sequence as shown in SEQ ID NOs 167, 175, 183, 191 and 199.

In one embodiment, the present invention is directed to an isolated polynucleotide encoding an antibody binding to DR5 of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable light chain sequence as shown in SEQ ID NOs 171, 179, 187, 195 and 203.

In another embodiment, the invention is directed to an isolated polynucleotide encoding a bispecific antibody of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable heavy chain sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence in SEQ ID NOs 167, 175, 183, 191, 199, 207 or 209.

In another embodiment, the invention is directed to an isolated polynucleotide encoding a bispecific antibody of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable lightchain sequence that is at least about 80%, 85%, 90%,

95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence in SEQ ID NOs 171, 179, 187, 195, 203, 208 or 210.

In another embodiment, the invention is directed to an isolated polynucleotide encoding an antibody binding to DR5 of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable heavy chain sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence in SEQ ID NOs 167, 175, 183, 191 or 199.

In another embodiment, the invention is directed to an isolated polynucleotide encoding an antibody binding to DR5 of the invention or a fragment thereof, wherein the polynucleotide comprises a sequence that encodes a variable light chain sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to an amino acid sequence in SEQ ID NOs 171, 179, 187, 195 or 203.

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

In further objects the present invention relates to an expression vector comprising a nucleic acid sequence of the present invention and to a prokaryotic or eukaryotic host cell comprising a vector of the present invention. In addition a method of producing an antibody comprising culturing the host cell so that the antibody is produced is provided.

G. Antibody Variants

In certain embodiments, amino acid sequence variants of the bispecific antibodies and antibodies binding to DR5 provided herein are contemplated, in addition to those described above. For example, it may be desirable to improve the binding affinity and/or other biological properties of the bispecific antibody or the antibody binding to DR5. Amino acid sequence variants of a bispecific antibody or an antibody binding to DR5 may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the bispecific antibody or the antibody binding to DR5, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

1. Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table B under the heading of "conservative substitutions." More substantial changes are provided in Table B under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

TABLE B

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

2. Glycosylation variants

In certain embodiments, a bispecific antibody or an antibody binding to DR5 provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the bispecific antibody or the antibody binding to DR5 comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH*

15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in a bispecific antibody or an antibody binding to DR5 of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, bispecific antibody variants or variants of antibodies binding to DR5 are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Bispecific antibodies variants or variants of antibodies binding to DR5 are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the bispecific antibody or the antibody binding to DR5 is bisected by GlcNAc. Such bispecific antibody variants or variants of antibodies binding to DR5 may have reduced

fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

3. Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered bispecific antibodies or antibodies binding to DR5, e.g., "thioMAbs," in which one or more residues of a bispecific antibody or antibodies binding to DR5 are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the bispecific antibody or the antibody binding to DR5. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

H. Recombinant Methods and Compositions

Bispecific antibodies and antibodies binding to DR5 of the invention may be obtained, for example, by solid-state peptide synthesis (e.g. Merrifield solid phase synthesis) or recombinant production. For recombinant production one or more polynucleotide encoding the bispecific antibodies or antibodies binding to DR5 (or fragments), e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such polynucleotide may be readily isolated and sequenced using conventional procedures. In one embodiment a vector, preferably an expression vector, comprising one or more of the polynucleotides of the invention is provided. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of a bispecific antibody (fragment) or an antibody (fragment) binding to DR5 along with appropriate transcriptional/translational control signals. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., MOLECULAR CLONING: A LABORATORY

MANUAL, Cold Spring Harbor Laboratory, N.Y. (1989); and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Associates and Wiley Interscience, N.Y (1989). The expression vector can be part of a plasmid, virus, or may be a nucleic acid fragment. The expression vector includes an expression cassette into which the polynucleotide encoding the bispecific antibody (fragment) or an antibody (fragment) binding to DR5 (i.e. the coding region) is cloned in operable association with a promoter and/or other transcription or translation control elements. As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, if present, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, 5' and 3' untranslated regions, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, e.g. on a single vector, or in separate polynucleotide constructs, e.g. on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, e.g. a vector of the present invention may encode one or more polypeptides, which are post- or co-translationally separated into the final proteins via proteolytic cleavage. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a polynucleotide encoding the bispecific antibody (fragment) or an antibody (fragment) binding to DR5 of the invention, or variant or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain. An operable association is when a coding region for a gene product, e.g. a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination

signals, can be operably associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein. A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions, which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (e.g. the immediate early promoter, in conjunction with intron-A), simian virus 40 (e.g. the early promoter), and retroviruses (such as, e.g. Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit â-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as inducible promoters (e.g. promoters inducible tetracyclins). Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from viral systems (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence). The expression cassette may also include other features such as an origin of replication, and/or chromosome integration elements such as retroviral long terminal repeats (LTRs), or adeno-associated viral (AAV) inverted terminal repeats (ITRs).

Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. For example, if secretion of the bispecific antibody or the antibody binding to DR5 is desired, DNA encoding a signal sequence may be placed upstream of the nucleic acid encoding a bispecific antibody of the invention or the antibody binding to DR5 of the invention or a fragment thereof. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the translated polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.* an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal

peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse β-glucuronidase.

DNA encoding a short protein sequence that could be used to facilitate later purification (e.g. a histidine tag) or assist in labeling the bispecific antibody or the antibody binding to DR5 may be included within or at the ends of the bispecific antibody (fragment) or the antibody (fragment) binding to DR5 encoding polynucleotide.

In a further embodiment, a host cell comprising one or more polynucleotides of the invention is provided. In certain embodiments a host cell comprising one or more vectors of the invention is provided. The polynucleotides and vectors may incorporate any of the features, singly or in combination, described herein in relation to polynucleotides and vectors, respectively. In one such embodiment a host cell comprises (e.g. has been transformed or transfected with) a vector comprising a polynucleotide that encodes (part of) a bispecific antibody or an antibody binding to DR5 of the invention. As used herein, the term "host cell" refers to any kind of cellular system which can be engineered to generate the bispecific antibodies or an antibody binding to DR5 of the invention or fragments thereof. Host cells suitable for replicating and for supporting expression of bispecific antibodies or of antibodies binding to DR5 are well known in the art. Such cells may be transfected or transduced as appropriate with the particular expression vector and large quantities of vector containing cells can be grown for seeding large scale fermenters to obtain sufficient quantities of the bispecific antibody or of the antibodies binding to DR5 for clinical applications. Suitable host cells include prokaryotic microorganisms, such as E. coli, or various eukaryotic cells, such as Chinese hamster ovary cells (CHO), insect cells, or the like. For example, polypeptides may be produced in bacteria in particular when glycosylation is not needed. After expression, the polypeptide may be isolated from the bacterial cell paste in a soluble fraction and can be further purified. In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for polypeptide-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized”, resulting in the production of a polypeptide with a partially or fully human glycosylation pattern. See Gerngross, Nat Biotech 22, 1409-1414 (2004), and Li et al., Nat Biotech 24, 210-215 (2006). Suitable host cells for the expression of (glycosylated) polypeptides are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant

cell cultures can also be utilized as hosts. See e.g. US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants). Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293T cells as described, e.g., in Graham et al., J Gen Virol 36, 59 (1977)), baby hamster kidney cells (BHK), mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol Reprod 23, 243-251 (1980)), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells (as described, e.g., in Mather et al., Annals N.Y. Acad Sci 383, 44-68 (1982)), MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including dhfr$^-$ CHO cells (Urlaub et al., Proc Natl Acad Sci USA 77, 4216 (1980)); and myeloma cell lines such as YO, NS0, P3X63 and Sp2/0. For a review of certain mammalian host cell lines suitable for protein production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003). Host cells include cultured cells, e.g., mammalian cultured cells, yeast cells, insect cells, bacterial cells and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue. In one embodiment, the host cell is a eukaryotic cell, preferably a mammalian cell, such as a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g., Y0, NS0, Sp20 cell).

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

In one embodiment, a method of producing a bispecific antibody or an antibody binding to DR5 according to the invention is provided, wherein the method comprises culturing a host cell comprising a polynucleotide encoding the bispecific antibody or the antibody binding to DR5, as provided herein, under conditions suitable for expression of the bispecific antibody or the antibody binding to DR5, and recovering the bispecific antibody or the antibody binding to DR5 from the host cell (or host cell culture medium).

The components of the bispecific antibody or the antibody binding to DR5 are genetically fused to each other. Bispecific antibodies or the antibodies binding to DR5 can be designed such that its components are fused directly to each other or indirectly through a linker sequence. The composition and length of the linker may be determined in accordance with methods well known in the art and may be tested for efficacy. Examples of linker sequences between different components of bispecific antibodies are found in the sequences provided herein. Additional sequences may also be included to incorporate a cleavage site to separate the individual components of the fusion if desired, for example an endopeptidase recognition sequence.

In certain embodiments the Fab fragments forming part of the bispecific antibody or the antibody binding to DR5 comprise at least an antibody variable region capable of binding an antigenic determinant. Variable regions can form part of and be derived from naturally or non-naturally occurring antibodies and fragments thereof. Methods to produce polyclonal antibodies and monoclonal antibodies are well known in the art (see e.g. Harlow and Lane, "Antibodies, a laboratory manual", Cold Spring Harbor Laboratory, 1988). Non-naturally occurring antibodies can be constructed using solid phase-peptide synthesis, can be produced recombinantly (e.g. as described in U.S. patent No. 4,186,567) or can be obtained, for example, by screening combinatorial libraries comprising variable heavy chains and variable light chains (see e.g. U.S. Patent. No. 5,969,108 to McCafferty).

Any animal species of antibody, antibody fragment, antigen binding domain or variable region can be used in the bispecific antibodies or the antibodies binding to DR5 of the invention. Non-limiting antibodies, antibody fragments, antigen binding domains or variable regions useful in the present invention can be of murine, primate, or human origin. If the bispecific antibody or the antibody binding to DR5 is intended for human use, a chimeric form of antibody may be used wherein the constant regions of the antibody are from a human. A humanized or fully human form of the antibody can also be prepared in accordance with methods well known in the art (see e. g. U.S. Patent No. 5,565,332 to Winter). Humanization may be achieved by various methods including, but not limited to (a) grafting the non-human (e.g., donor antibody) CDRs onto human (e.g. recipient antibody) framework and constant regions with or without retention of critical framework residues (e.g. those that are important for retaining good antigen binding affinity or antibody functions), (b) grafting only the non-human specificity-determining regions (SDRs or a-CDRs; the residues critical for the antibody-antigen interaction) onto human framework and constant regions, or (c) transplanting the entire non-human variable domains, but "cloaking" them with a human-like section by replacement of surface residues. Humanized antibodies and

methods of making them are reviewed, e.g., in Almagro and Fransson, Front Biosci 13, 1619-1633 (2008), and are further described, e.g., in Riechmann et al., Nature 332, 323-329 (1988); Queen et al., Proc Natl Acad Sci USA 86, 10029-10033 (1989); US Patent Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Jones et al., Nature 321, 522-525 (1986); Morrison et al., Proc Natl Acad Sci 81, 6851-6855 (1984); Morrison and Oi, Adv Immunol 44, 65-92 (1988); Verhoeyen et al., Science 239, 1534-1536 (1988); Padlan, Molec Immun 31(3), 169-217 (1994); Kashmiri et al., Methods 36, 25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, Mol Immunol 28, 489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., Methods 36, 43-60 (2005) (describing "FR shuffling"); and Osbourn et al., Methods 36, 61-68 (2005) and Klimka et al., Br J Cancer 83, 252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human antibodies and human variable regions can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr Opin Pharmacol 5, 368-74 (2001) and Lonberg, Curr Opin Immunol 20, 450-459 (2008). Human variable regions can form part of and be derived from human monoclonal antibodies made by the hybridoma method (see e.g. Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)). Human antibodies and human variable regions may also be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge (see e.g. Lonberg, Nat Biotech 23, 1117-1125 (2005). Human antibodies and human variable regions may also be generated by isolating Fv clone variable region sequences selected from human-derived phage display libraries (see e.g., Hoogenboom et al. in Methods in Molecular Biology 178, 1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001); and McCafferty et al., Nature 348, 552-554; Clackson et al., Nature 352, 624-628 (1991)). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments.

In certain embodiments, the Fab fragments useful in the present invention are engineered to have enhanced binding affinity according to, for example, the methods disclosed in U.S. Pat. Appl. Publ. No. 2004/0132066, the entire contents of which are hereby incorporated by reference. The ability of the bispecific antibody or the antibody binding to DR5 of the invention to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance technique (analyzed on a BIACORE T100 system) (Liljeblad, et al., Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)).

Competition assays may be used to identify an antibody, antibody fragment, antigen binding domain or variable domain that competes with a reference antibody for binding to a particular antigen. In certain embodiments, such a competing antibody binds to the same epitope (e.g. a linear or a conformational epitope) that is bound by the reference antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ). In an exemplary competition assay, immobilized antigen is incubated in a solution comprising a first labeled antibody that binds to the antigen and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to the antigen. The second antibody may be present in a hybridoma supernatant. As a control, immobilized antigen is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to the antigen, excess unbound antibody is removed, and the amount of label associated with immobilized antigen is measured. If the amount of label associated with immobilized antigen is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to the antigen. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Bispecific antibodies or antibodies binding to DR5 prepared as described herein may be purified by art-known techniques such as high performance liquid chromatography, ion exchange chromatography, gel electrophoresis, affinity chromatography, size exclusion chromatography, and the like. The actual conditions used to purify a particular protein will depend, in part, on factors such as net charge, hydrophobicity, hydrophilicity etc., and will be apparent to those having skill in the art. For affinity chromatography purification an antibody, ligand, receptor or antigen can be used to which the bispecific antibody or the antibody binding to DR5 binds. For example, for affinity chromatography purification of bispecific antibodies of the invention, a matrix with protein A or protein G may be used. Sequential Protein A or G affinity chromatography and size exclusion chromatography can be used to isolate a bispecific antibody essentially as described in the Examples. The purity of the bispecific antibody or the antibody binding to DR5 can be determined by any of a variety of well known analytical methods including gel electrophoresis, high pressure liquid chromatography, and the like.

I. Assays

Bispecific antibodies that bind to DR5 and FAP and antibodies binding to DR5 provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art

1. Affinity assays

The affinity of the bispecific antibody and the antibody binding to DR5 provided therein for DR5 and/ or FAP can be determined in accordance with the methods set forth in the Examples by surface plasmon resonance (SPR), using standard instrumentation such as a BIAcore instrument (GE Healthcare), and receptors or target proteins such as may be obtained by recombinant expression. Alternatively, binding of bispecific antibody and the antibody binding to DR5 provided therein to DR5 and/or FAP may be evaluated using cell lines expressing the particular receptor or target antigen, for example by flow cytometry (FACS). A specific illustrative and exemplary embodiment for measuring binding affinity is described in the following and in the Examples below.

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

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

To determine the affinity to the target antigen, bispecific constructs are captured by an anti human Fab specific antibody (GE Healthcare) that is immobilized on an activated CM5-sensor chip surface as described for the anti Penta-His antibody. The final amount of coupled protein is is approximately 12000 RU. The bispecific constructs are captured for 90 s at 300 nM. The

target antigens are passed through the flow cells for 180 s at a concentration range from 250 to 1000 nM with a flowrate of 30 µl/min. The dissociation is monitored for 180 s.

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

2. Binding assays and other assays

In one aspect, a bispecific antibody or an antibody that binds to DR5 of the invention is tested for its antigen binding activity, e.g., by known methods such as ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with a specific anti-FAP antibody or a specific anti-DR5 antibody for binding to FAP or DR5 respectively. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by a specific anti-FAP antibody or a specific anti-DR5 antibody. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). Further methods are described in the example section.

3. Activity assays

In one aspect, assays are provided for identifying bispecific antibodies that bind to DR5 and FAP or antibodies that binds to DR5 thereof having biological activity. Biological activity may include, e.g., DNA fragmentation, induction of apoptosis and lysis of targeted cells. Antibodies having such biological activity in vivo and/or in vitro are also provided.

In certain embodiments, a bispecific antibody or an antibodiy that binds to DR5 of the invention is tested for such biological activity. Assays for detecting cell lysis (e.g. by measurement of LDH release) or apoptosis (e.g. using the TUNEL assay) are well known in the art. Assays for measuring ADCC or CDC are also described in WO 2004/065540 (see Example 1 therein), the entire content of which is incorporated herein by reference.

J. Pharmaceutical Formulations

Pharmaceutical formulations of a bispecific antibody that binds to DR5 and FAP or an antibody that binds to DR5 as described herein are prepared by mixing such bispecific antibody or antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

K. Therapeutic Methods and Compositions

Any of the bispecific antibodies that bind to DR5 and FAP and the novel antibodies binding to DR5 provided herein may be used in therapeutic methods.

In one aspect, a bispecific antibody that binds to DR5 and FAP for use as a medicament is provided. In further aspects, a bispecific antibody that binds to DR5 and FAP use in treating cancer is provided. In certain embodiments, a bispecific antibody that binds to DR5 and FAP for use in a method of treatment is provided. In certain embodiments, the invention provides a bispecific antibody that binds to DR5 and FAP for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the bispecific antibody that binds to DR5 and FAP. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. An “individual” according to any of the above embodiments is preferably a human. In one preferred embodiment said cancer is pancreatic cancer or colorectal carcinoma.

In one aspect, an antibody that binds to DR5 for use as a medicament is provided. In further aspects, a antibody that binds to DR5 use in treating cancer is provided. In certain embodiments, a antibody that binds to DR5 for use in a method of treatment is provided. In certain embodiments, the invention provides an antibody that binds to DR5 for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the antibody that binds to DR5. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. An “individual” according to any of the above

embodiments is preferably a human. In one preferred embodiment said cancer is pancreatic cancer or colorectal carcinoma.

In a further aspect, the invention provides for the use of a bispecific antibody that binds to DR5 and FAP in the manufacture or preparation of a medicament. In another aspect, the invention provides for the use of an antibody that binds to DR5 in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer. In a further embodiment, the medicament is for use in a method of treating cancer comprising administering to an individual having cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for treating cancer. In one embodiment, the method comprises administering to an individual having cancer an effective amount of a bispecific antibody that binds to DR5 and FAP or of a novel antibody binding to DR5. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human. In one preferred embodiment said cancer is pancreatic cancer or colorectal carcinoma.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the bispecific antibodies that bind to DR5 and FAP provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the bispecific antibodies that bind to DR5 and FAP provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the bispecific antibodies that bind to DR5 and FAP provided herein and at least one additional therapeutic agent, e.g., as described below.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the antibodies that bind to DR5 provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the antibodies that bind to DR5 provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the antibodies that bind to DR5 provided herein and at least one additional therapeutic agent, e.g., as described below.

A bispecific antibody or a novel antibody binding to DR5 of the invention can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Bispecific antibodies or novel antibodies binding to DR5 of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The bispecific antibody or the novel antibody binding to DR5 need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of a bispecific antibody or a novel antibody binding to DR5 of the invention will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the bispecific antibody or the novel antibody binding to DR5 is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the bispecific antibody or to the novel antibody binding to DR5, and the discretion of the attending physician. The bispecific antibody or the novel antibody binding to DR5 is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of the bispecific antibody or the novel antibody binding to DR5 can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on

the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the bispecific antibody or the novel antibody binding to DR5 would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (*e.g.* such that the patient receives from about two to about twenty, or *e.g.* about six doses of the bispecific antibody or of the novel antibody binding to DR5). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to a bispecific antibody that binds to DR5 and FAP or a novel antibody binding to DR5 of the invention.

L. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a bispecific antibody or a novel antibody binding to DR5 of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a bispecific antibody or a novel antibody binding to DR5 of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection

(BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to a bispecific antibody that binds to DR5 and FAP or a novel antibody binding to DR5 of the invention.

III. EXAMPLES

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

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Example 1: A DR5 – FAP death receptor agonistic bispecific antibody is able to mediate apoptosis of one cell line via cross-linking by a second cell line.

One approach of induction of apoptosis by cross-linking of death receptors as DR5 (apart from cross-linking via an antigen expressed by the tumor cell), is targeting the stroma surrounding the tumor. In that case the targeted antigen is not displayed directly by the tumor cells but by a second, different cell type. One example for this kind of antigen would be FAP (fibroblast activation protein). This protein is expressed on activated fibroblast as they are found in the tumor stroma.

To investigate the possibilities of tumor targeted induction of apoptosis using bispecific death receptor agonistic antibodies targeting human DR5 and an antigen from the tumor stroma, bispecific molecules were generated that consist of an IgG1 part that recognizes DR5 and a FAP binding scFv that is fused to the C-terminus of the heavy chain of the antibody. The sequence of the DR5 targeting IgG was taken from the Drozitumab sequence as described in US2007 / 0031414 A1. The sequence of variable heavy and light chain of the FAP binding scFv moieties were taken from Fab anti FAP molecules (3F2 or 4G8) isolated by phage display as described in WO2012/020006A1. The FAP scFvs are fused by a $(G_4S)_2$ connector to the C-terminus of the anti DR5 IgG heavy chain or light chain. Both anti FAP antibodies bind to different epitopes on

fibroblast activation protein (FAP) and are cross-reactive with the human, murine and cynomolgus antigens.

Table 1: Description of tested bispecific DR5-FAP molecules with C-terminal fusion of FAP binding scFv's to DR5 heavy or light chain. Linker and connector length and purification yields are given.

Molecule	Description	Connector	Linker in scFv	Yield [mg/L]
3F2-scFv_HC (SEQ ID NO.:111)	Fusion of disulfide stabilized scFv (H44L100) to C-terminus of Drozitumab heavy chain	$(G_4S)_2$	$(G_4S)_4$	2.53
4G8-scFv_HC (SEQ ID NO.:112)	Fusion of disulfide stabilized scFv (H44L100) to C-terminus of Drozitumab heavy chain	$(G_4S)_2$	$(G_4S)_4$	4.29
4G8-scFv_LC (SEQ ID NO.:113)	Fusion of disulfide stabilized scFv (H44L100) to C-terminus of Drozitumab light chain	$(G_4S)_2$	$(G_4S)_4$	2.15

In this kind of setting two different cell lines have to be used for the *in vitro* activity assays: one cell line (the target cell line) should express human DR5, has to be apoptosis competent but does not need to express FAP. The second cell line (the effector cell line) has to be apoptosis negative (either by apoptosis resistance or by not expressing DR5) but needs to express FAP on the surface.

One possible effector cell line that fulfills the desired criteria is the human fibroblast cell line GM05389. As shown in figure 1a this cell line expresses significant levels of FAP compared to the cell line SW872 which only showed FAP expression with the highest tested antibody concentration (10 µg / ml) but does not undergo apoptosis by non-cross-linked Drozitumab as seen in figure 1b. Therefore this cell line seems to be a potential effector cell line in an apoptosis assay where DNA fragmentation of a target cell line is induced by cross-linking via an antigen expressed on a second cell line.

As a target cell line the human kidney-adenocarcinoma cell line ACHN or the human breast cancer cell line MDA-MB-231 were used. They express comparable, low levels of DR5 (Figure 2) and are sensitive to DR5 mediated apoptosis induction. In figure 3 the results of

induction of DNA fragmentation of ACHN cells compared to the combination of ACHN and GM05389 cell lines by tumor targeted cross-linking of DR5 via FAP is summarized. The control antibody (Drozitumab) induces apoptosis upon cross-linking via a secondary antibody that targets the Fc part (white bars) in both cell lines, but shows also some activity without crosslinking. The bispecific DR5-FAP molecules show significant induction of apoptosis in the presence of both – target and effector cell line (black bars). In the absence of FAP expressing fibroblasts (GM05389) there is a slightly increased apoptosis compared to non-cross-linked Drozitumab (grey bars). We interpret this result in a way that the DR5 receptors on ACHN cells are cross-linked upon binding to the FAP antigen expressed by the fibroblast cell line GM05389. All molecules show comparable maximal apoptosis activity.

Example 2: DR5 bispecific agonistic antibodies with cross-reactive FAP binders in scFab format fused to Drozitumab demonstrate apoptosis activity in co-culture assays

As demonstrated in example 1 DR5-FAP bispecific agonistic antibodies in which the FAP targeting moiety in scFv format is fused to the C-terminus of the Drozitumab heavy or light chain (2x2 format) are able to induce apoptosis in a two cell line co-culture setting in which one cell line expresses FAP (effector cells) while the second cell line serves as the DR5 receptor target.

Since these molecules containing an scFv fusion showed some disadvantageous properties (low production yield and tendency to form aggregates), constructs were generated in which the FAP binding unit was replaced by single chain Fab entities (scFab), fused to the C-terminus of either the heavy or light chain of Drozitumab leading to the production of four different molecules as described in table 2. The connection of the scFab units to the IgG part of the bispecific molecules occurs via a $(G_4S)_4$ sequence whereas the scFab internal linker consists of 32 amino acids.

These molecules were transiently produced by standard recombinant technologies and purified in sufficient amounts and good quality for detailed testing of FAP and DR5 binding (FACS; Biacore, not shown).

Table 2: Description and characterization of the different Drozitumab-FAP (scFab) fusions. In all molecules the anti FAP scFab is fused by a 20 mer connector ($(G_4S)_4$) to Drozitumab.

Name	Description	Yield [mg / L]	Connector

GA803_A01_B01A_004 **3F2_scFab_HC** (SEQ ID NO.:114)	scFab: 3F2; VLCL-VHCH1 C-terminal fusion to Drozitumab heavy chain (2x2)	3.9	$(G_4S)_4$
GA803_A01_B01B_005 **3F2_scFab_LC** (SEQ ID NO.:115)	scFab: 3F2; VLCL-VHCH1 C-terminal fusion to Drozitumab light chain (2x2)	4.9	$(G_4S)_4$
GA803_A01_B02A_001 **4G8_scFab_HC** (SEQ ID NO.:116)	scFab: 4G8; VLCL-VHCH1 C-terminal fusion to Drozitumab heavy chain (2x2)	2.0	$(G_4S)_4$
GA803_A01_B02B_002 **4G8_scFab_LC** (SEQ ID NO.:117)	scFab: 4G8; VLCL-VHCH1 C-terminal fusion to Drozitumab light chain (2x2)	2.5	$(G_4S)_4$

Analysis of apoptosis induction of a DR5 expressing target cell line (e.g. the renal carcinoma cell line ACHN) in the presence and absence of a second, FAP expressing 'effector' cell line (e.g. fibroblast line GM05389) is shown in figure 4a and 4b.The apoptosis induction was analysed with a DNA fragmentation assay. In the assays a ratio of target to effector cells of 1 : 1 was used and DNA fragmentation after 24 hrs of co-culture in the presence of Drozitumab, hyper-cross-linked Drozitumab or the bispecific constructs (all used in concentrations of 1 µg / ml and 0.1 µg / ml) was analyzed.

For all tested constructs it could be demonstrated that the bispecific molecules, independent of the used FAP binder (either 3F2 or 4G8) as scFabs fused to Drozitumab show increased apoptosis induction in ACHN target cells only in the presence of the fibroblast effector cell line. However, at high concentrations of the bispecific molcules a low degree of apoptosis activity could be observed in the absence of FAP expressing fibroblasts. In general and independent on the used FAP binder, molecules with scFab fusion to the C-terminus of the heavy chain were more active as molecules where the scFabs were fused to the C-terminus of the light chain. In addition, 4G8 containing constructs seemed to be more potent than 3F2 containing

molecules. However, activity of scFab based formats was lower compared to the analogous scFv based constructs.

Drozitumab without additional cross-linking by anti Fc antibodies showed a low apoptosis induction. However, Drozitumab hyper-cross-linked via a secondary anti Fc antibody alone - a highly artificial situation - revealed the highest apoptosis activity which could not been reached by any of the tested molecules.

Example 3: DR5 - FAP bispecific agonistic antibodies in CrossFab format demonstrate superior characteristics over scFab containing molecules

Since the evaluated scFab containing bispecific molecules still showed some disadvantageous characteristics (e.g. low expression yield, optimizable apoptosis activity) a novel format that should overcome these liabilities was analyzed: fusion of a FAP binder (4G8) in CrossFab format to the C-terminus of Drozitumab heavy chain. In this format the FAP binding moiety is used in a 'crossover' exchange of variable regions in the Fab fragment as described in table 3 and shown in figure 5. The CrossFab molecules are linked via a 20mer connector ($(G_4S)_4$) to the Drozitumab heavy chain.

Table 3: Description and production yields of bispecific Drozitumab – FAP CrossFab constructs in two different formats.

Figure	Name	Description	Yield [mg / L] Monomer [%]
5a	GA803_A01_E02A_014 **Drozitumab–X – FAP_A** (2+2) (SEQ ID NO.:118, 119,120)	• Fusion of 4G8 VH-CL to C-terminus of Drozitumab heavy chain (SEQ ID NO.:118) • Separate VL-CH1 cassette (SEQ ID NO.:120) • Separate Drozitumab light chain cassette (SEQ ID NO.:119)	36.1 100.0
5b	GA803_A01_E02A_015	• Fusion of 4G8 VL-CH1 to C-terminus of Drozitumab	16.7

	Drozitumab–X – FAP_B (2+2) (SEQ ID NO.:121, 122, 119)	heavy chain (SEQ ID NO.:121) • Separate VH-CL cassette (SEQ ID NO.:122) • Separate Drozitumab light chain cassette (SEQ ID NO.:119)	100.0

Both CrossFab molecules (also see figure 5 for organization of the constructs) were transiently produced in HEK293 EBNA cells (either in adherent or suspension cells) and purified with standard methods. Compared to scFab and scFv containing bispecific constructs the CrossFab molecules exhibited significantly increased expression levels leading to approximately 10-fold higher product yields with acceptable low aggregate contents as shown in table 4.

Figure 6 demonstrates the aggregate content during production of different bispecific DR5-FAP molecules. Chromatograms from preparative size exclusion chromatography during purification are shown for the scFv (A), scFab (B) and CrossFab (C) fusion to the C-terminus of Drozitumab heavy chain. The scFv and scFab fusion constructs show significantly higher aggregate contents compared to the CrossFab containing molecule. Removal of these aggregates during purification leads to huge loss of material which can easily be seen from the yields obtained after purification.

Binding of the molecules to human FAP, expressed on recombinant HEK293 cells was detected by FACS analysis. Figure 7 shows the FACS binding results of two Drozitumab-CrossFab molecules (Drozitumab–X–FAP_A, dotted bar and Drozitumab–X–FAP_B, white bar) compared to an analogous scFab construct (hatched bar) or the corresponding FAP (4G8) IgG molecule (black bar). All three bispecific molecules in which the 4G8 FAP binding moiety is fused to the C-terminus of the Drozitumab heavy chain are binding similar or in a similar range to the human FAP expressed on the membranes of recombinant HEK cells as the IgG construct indicating that this fusion position does not have an N-terminal blocking effect on the tested FAP binder. The used negative control molecules in this assay (secondary detection antibody and Drozitumab) do not bind in a detectable manner to FAP expressing HEK cells.

Table 4: Comparison of yield and quality of all different tested bispecific DR5-FAP molecules.

	Molecule	Yield [mg/L]	Aggregates [%] before	after SEC	Low molecular weight content [%]	Monomer [%]
1	3F2 scFv_HC (SEQ ID NO.:111)	2.53	59	0.00	4.13	96.34
2	4G8 scFv_HC (SEQ ID NO.:112)	4.29	n.d.	0.00	1.50	98.50
3	4G8 scFv_LC (SEQ ID NO.:113)	2.15	73	0.00	0.00	100.00
4	3F2 scFab_HC (SEQ ID NO.:114)	3.99	72	0.00	0.00	100.00
5	3F2 scFab_LC (SEQ ID NO.:115)	4.99	n.d.	0.00	0.00	100.00
6	4G8 scFab_HC (SEQ ID NO.:116)	2.03	78	0.00	0.00	100.00
7	4G8 scFab_LC (SEQ ID NO.:117)	2.47	66	0.00	0.00	100.00
8	4G8-X-Fab_A (SEQ ID NO.:118, 119,120)	36.10	90	0.00	0.00	100.00
9	4G8-X-Fab_B (SEQ ID NO.:121, 122, 119)	16.67	94	0.00	0.00	100.00

In figure 8 the results of Surface Plasmon Resonance analysis (SPR, Biacore) are shown in which the simultaneous binding of the bispecific CrossFab molecules to DR5 and FAP was evaluated. For this assay the antigen (human DR5 as Fc fusion, SEQ ID NO.:316) was coupled to the Biacore chip followed by injection of the CrossFab molecules as first analyte. After binding of the scFab molecules to DR5, recombinant soluble human or murine FAP (SEQ ID NO.:156 and 157) was used as the second analyte. For all tested bispecific molecules concentration dependent simultaneous binding to DR5 and human and murine FAP was demonstrated, as indicated by the increase of the overall response rate after injection of the first analyte, obtained upon injection of the second analyte. Both tetravalent bispecific CrossFab

molecules (2x2; Drozitumab-X-FAP_A and Drozitumab-X-FAP_B) showed similar binding to DR5 and human and murine FAP (figure 8; A-D).

Figure 9 shows the induction of apoptosis as determined by DNA fragmentation assay after 24 hrs of Drozitumab vs. two different bispecific Drozitumab–X–FAP molecules and one Drozitumab_4G8_scFab construct on the breast carcinoma cell line MDA-MB-231 in the absence or presence of FAP expressing fibroblast cells GM05389. Under the applied conditions, hyper-cross-linked Drozitumab and the bispecific DR5 – FAP molecules exhibit concentration dependent induction of apoptosis. While the bispecific constructs at low concentrations seemed to be dependent on the so-called bystander apoptosis (apoptosis activity only with DR5 and FAP expressing cell lines present) they also induced apoptosis of MDA-MB-231 cells alone at high concentrations. Hyper-cross-linked Drozitumab induced high levels of apoptosis also at low concentrations when only MDA-MB-231 cells were present. Both bispecific molecules exhibited maximal apoptosis induction already at a concentration of 0.7 nM.

To test the specificity of the bispecific constructs for their apoptosis induction activity being dependent on cross-linking via FAP a different assay set up was chosen (figure 10). In this setting recombinant human FAP or an unrelated control protein (white bars) were coated onto ELISA plates. These proteins were incubated with the bispecific molecules or the relevant controls before target cells (MDA-MB-231) were added and incubated for 24 hrs. A concentration dependent DNA fragmentation indicative for apoptosis induction could be demonstrated for the hyper-cross-linked Drozitumab (grey bars), a bispecific scFab molecule (hatched bars) and the bispecific CrossFab molecules (black bars) as shown in figure 10. While apoptosis induction with cross-linked Drozitumab always was in the same range with FAP or the control protein coated (over the entire tested concentration range) the apoptosis activity of CrossFab molecules was higher when FAP was coated onto the plates compared to the control protein. At concentrations of 0.7 nM and below significant apoptosis only could be detected in the presence of coated FAP, indicating specific cross-linking of DR5 on the target MDA-MB-231 cells. The bispecific Drozitumab-FAP constructs containing the FAP moiety fused as a CrossFab to the C-terminus of the Drozitumab heavy chain exhibit superior apoptosis induction over Drozitumab alone at the tested concentrations for different tumor cell lines analyzed in co-culture apoptosis assays (DNA fragmentation assay) over 24 hrs as shown in figure 11. In this assay FAP expressing fibroblasts (GM05389) were co-cultured with a series of different tumor cell lines (MDA-MB-231, breast cancer, black bars; U-87MG, glioblastoma, grey bars; FaDu, head and neck, white bars and A549, lung cancer, hatched bars) in the presence of either Drozitumab, Drozitumab cross-linked with anti-Fc antibody, or Drozitumab-X-FAP construct (all at concentrations of 7 nM and 0.7 nM). Apoptosis induction of cross-linked Drozitumab at a concentration of 7 nM was set to 100 % and the activities of the other tested molecules were calculated accordingly. While Drozitumab alone at a concentration of 7 nM showed up to 50 %

activity (depending on the tested tumor cell line), the CrossFab molecule exhibited apoptosis induction in the range of 75 – 90 % of hyper cross-linked Drozitumab with all tested cell lines. Drozitumab alone at 0.7 nM demonstrated low activity while the bispecific molecule displayed significant apoptosis induction activity (up to 100 % with FaDu cells, between 60 and 90 % with the other tested cell lines).

Example 4: Preparation of antigens and screening tools for the generation of novel DR5 binders

Due to some suboptimal properties of Drozitumab in bispecific format (low productivity, active without cross-linking, N-terminal fusion inactivates the antibody) new DR5 antibodies were isolated which overcome these liabilities. For generation of suitable antigens to be used in isolation of novel DR5 binders and for screening and selection of those, a series of fusion proteins have been constructed. From each receptor gene the extracellular domain (ECD) encoding region was amplified by PCR and fused to a generic protein partner to generate the following formats (as depicted in figure 12):

1. Extracellular domain (ECD) fused in frame to Avi-tag and Hexahis-tag

2. ECD fused to Fc of human IgG1 consisting of the hinge region and CH2 and CH3 domains followed by an Avi tag. Between ECD and Fc an AcTev protease cleavage site was inserted (--(resulting in a dimeric antigen)

3. As in 2 but the ECD is fused to an Fc with knob-into-hole mutations. The antigen-Fc fusion is co-expressed with the Fc-hole counterpart to obtain monomeric antigens

While the sequences of human DR5 and murine DR5 were known and annotated in the SwissProt database, the sequence of the cynomolgus homolog has not been described there. Based on homologies among human and rhesus DR5 gene sequences primers have been designed that were used for isolation of the cynomolgus antigen from RNA prepared from cynomolgus PBMCs. In brief, RNA was isolated from freshly isolated cynomolgus blood using the RNeasy Kit from Qiagen. After DNAseI digestion and elution of the RNA the OneStep RT-PCR kit (cDNA synthesis and amplification) from Qiagen (catalog number 210212) was used to amplify the cynomolgus DR5 gene with GAB-4039 (GCTGGCTCCTGGACTTCCATTTCC, SEQ ID NO 163) and GAB4040 (GACCCAGGGAGGCGCGGGGAG; SEQ ID NO 164) as primers, designed according to the known rhesus DR5 sequence. The PCR product was cloned into pCR2.1 Topo (Invitrogen) for sequencing. Analysis of the sequence revealed 89% homology to the human DR5 extracellular domain. Using the primers GAB-4145 (GTGCATTCCATCACCCGACAATCCCTAGATCCCCAGCG; SEQ ID NO 165) and GAB-

4146 (GCGTCGACTGATTCTTTGTGGACACACTCAATGTCAC; SEQ ID NO 166) the extracellular domain of the cynomolgus DR5 gene was cloned into a generic expression vector in fusion with human Fc.

Expression of the desired genes occurs under control of a MPSV promoter. In addition a 3' polyadenylation site is included and an oriP sequence for stable maintenance of the plasmids in EBV nuclear antigen (EBNA) expressing HEK293 cells.

For production of the antigens relevant expression vectors were transfected into HEK293 EBNA cells (either Ca_2PO_4 mediated or PEI dependent transfection). After 5 – 7 days of cultivation supernatants were harvested and purified via Protein A binding (Fc containing antigens) or by NI^{2+} affinity chromatography (Histag containing molecules) and subsequent size exclusion chromatography (SEC). If necessary the proteins were biotinylated via the C-terminal Avi tag. This could either be performed *in vivo*, by co-transfection / co-expression of a birA encoding plasmid or after purification of the antigen *in vitro* using a biotinylation kit from Avidity Cat No. BIRA. The following antigens were produced by transient gene expression in HEK293 EBNA:

Table 5: Antigen constructs and screening tools for isolation of antibodies against human DR5

#	Antigen	Amino acid	Format	Source	Acc. No
1	human DR5	56 – 207	ECD – Avi-his	Open Biosystems BC001281.1	O14763
2	human DR5	56 – 207	ECD – AcTev – hu Fc – Avi	as above	O14763
3	human DR5	56 – 207	ECD – AcTev – hu Fc knob – Avi	as above	O14763
4	murine DR5	53 – 153	ECD – AcTev – hu Fc – Avi	Open Biosystems BC065141.1	Q9QZM 4
5	cynom. DR5	58 – 185	ECD – AcTev – hu Fc – Avi	described herein and WO 2004/101608 A2	SEQ ID NO. 317

n.a.: not applicable

Recombinant human DR4-Fc (Cat No. 347-DR/CF), DcR1-Fc (Cat No. 630-TR/CF), DcR2-Fc /Cat No. 633-TR/CF) and OPG-Fc (Cat No. 805-=S/CF) were purchased from R&D Biosystems.

Example 5: Isolation of novel anti DR5 binders from generic Fab libraries

Antibodies with specificity for human DR5 were selected from a generic phage-displayed antibody library in the Fab format (DP47-3). This library was constructed on the basis of human germline genes using the V-domain pairing Vk3_20 (kappa light chain) and VH3_23 (heavy chain) comprising randomized sequence space in CDR3 of the light chain (L3) and CDR3 of the heavy chain (H3). Library generation was performed by assembly of 3 PCR-amplified fragments by splicing by overlapping extension (SOE) PCR. Fragment 1 comprises the the 5' end of the antibody gene including randomized L3, fragment 2 is a central constant fragment spanning from L3 to H3 whereas fragment 3 comprises randomized H3 and the 3' portion of the antibody gene. The following primer combinations were used to generate these library fragments for DP47-3 library: fragment 1 (LMB3 – LibL1b_new), fragment 2 (MS63 – MS64) and fragment 3 (Lib2H - fdseqlong), respectively. PCR parameters for production of library fragments were 5 min initial denaturation at 94 °C, 25 cycles of 1 min 94 °C, 1 min 58 °C, 1 min 72 °C and terminal elongation for 10 min at 72 °C. For assembly PCR, using equimolar ratios of the 3 fragments as template, parameters were 3 min initial denaturation at 94 °C and 5 cycles of 30 s 94 °C, 1 min 58 °C, 2 min 72 °C. At this stage, outer primers were added and additional 20 cycles were performed prior to a terminal elongation for 10 min at 72 °C. After assembly of sufficient amounts of full length randomized Fab constructs, they were digested *Nco*I / *Not*I alongside with similarly treated acceptor phagemid vector. 22.8 µg of Fab library were ligated with 16.2 µg of phagemid vector. Purified ligations were used for 68 transformations to obtain a final library size of 4.2×10^{10}. Phagemid particles displaying the Fab library were rescued and purified by PEG/NaCl purification to be used for selections.

Selections were carried out against HEK293-expressed monomeric or dimeric human DR5 fused to the Fc-portion of a human IgG1 antibody. For the generation of the monomeric antigen, the Fc knob-into-holes format was applied for heterodimerization of two different CH2-CH3 chains (only one of which carrying human DR5 ectodomain as N-terminal fusion). The antigens were enzymatically biotinylated via an avi-tag. Panning rounds were performed in solution according to the following pattern: 1. Preclearing of ~ 10^{12} phagemid particles using hu IgG1 coated at 10 µg/ml onto NUNC maxisorp plates to avoid Fc-binders, 2. binding of non-Fc binding phagemid particles from the supernatant of the pre-clearing reaction to 100 nM biotinylated human DR5 for 0.5 h in a total volume of 1 ml, 3. capture of biotinylated hu DR5 and attached specifically binding phage by incubation on neutravidin-coated microtiter plates for 10 min, 4. washing of beads using 5x 1 ml PBS/Tween20 and 5x 1 ml PBS, 5. elution of phage particles by addition of 1ml 100 mM TEA (triethylamine) for 10 min and neutralization by addition of 500 µl 1M Tris/HCl pH 7.4, 6. post-clearing step of eluted phage particles on human DcR2 to avoid cross-reactive binders and 7. re-infection of log-phase *E. coli* TG1 cells with the phage particles in the supernatant, infection with helperphage VCSM13 and subsequent

PEG/NaCl precipitation of phagemid particles to be used in subsequent selection rounds. Selections were carried out over 3 rounds using constant antigen concentrations at 100nM. In round 2, capture of antigen: phage complexes was performed by addition of 5.4×10^7 streptavidin-coated magnetic beads for 10 min instead of capture on neutravidin-coated microtiter plates. Specific binders were identified by ELISA as follows: 100 µl of 50 nM biotinylated human DR5 or DcR2 per well were coated on neutravidin plates. Moreover, 10 µg/ml human IgG1 were coated on NUNC maxisorp plates. Fab-containing bacterial supernatants were added and binding Fabs were detected via their Flag-tags by using an anti-Flag/HRP secondary antibody. Clones exhibiting signals on human DR5 but none on human DcR2 and human IgG1 were short-listed for further analyses.

Affinity (K_D) of selected Fab clones was measured by surface plasmon resonance using a ProteOn XPR36 instrument (Biorad) at 25°C with biotinylated mono- or bivalent DR5 antigens immobilized on NLC chips by neutravidin capture. Immobilization of antigens (ligand): Recombinant antigens were diluted with PBST (10 mM phosphate, 150 mM sodium chloride pH 7.4, 0.005% Tween 20) to 10 µg/ml, then injected at 30 µl/minute at varying contact times, to achieve immobilization levels of 200, 400 or 800 response units (RU) in vertical orientation. Injection of analytes: For one-shot kinetics measurements, injection direction was changed to horizontal orientation, two-fold dilution series of purified Fab (varying concentration ranges between 100 and 3.125 nM) were injected simultaneously at 50, 60 or 100µl/min along separate channels 1-5, with association times of 120, 180 or 200s, and dissociation times of 200 or 240s. Buffer (PBST) was injected along the sixth channel to provide an “in-line” blank for referencing. Association rate constants (k_{on}) and dissociation rate constants (k_{off}) were calculated using a simple one-to-one Langmuir binding model in ProteOn Manager v3.1 software by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) was calculated as the ratio k_{off}/k_{on}. Regeneration was performed in horizontal orientation using 50mM NaOH at a flow rate of 100 µl/min for a contact time of 18s.

Example 6: Selected DR5 binders are capable of inducing apoptosis upon cross-linking

To identify DR5 binders which are able to induce apoptosis of selected target cells only upon cross-linking the antibodies isolated from a Fab library were converted into the corresponding hu IgG1 format. In brief, the variable heavy and variable light chains of 46 unique DR5 binders from phage display were amplified by standard PCR reactions using the Fab clones as the template. The PCR products were purified and inserted (either by restriction endonuclease and ligase based cloning, or by ‘recombineering’ using the InFusion kit from Invitrogen) into suitable expression vectors in which they are fused to the appropriate human constant heavy or human constant light chain. The expression cassettes in these vectors consist of a chimeric

MPSV promoter and a synthetic polyadenylation site. In addition, the plasmids contain the oriP region from the Epstein Barr virus for the stable maintenance of the plasmids in HEK293 cells harboring the EBV nuclear antigen (EBNA). Antibodies were transiently produced in 50 ml scale in HEK293 EBNA cells as described. For a fast and high throughput purification, supernatants were neutralized and incubated with ProteinA Sepharose Fast Flow beads (GE Healthcare Cat No. 17-5138-01) for 16h. The supernatant/bead mixture was then passed over an empty, equilibrated PD-10 column (GE Healthcare Cat No. 17-0435-01) by gravity flow. The retained beads were washed twice and the antibody eluted with a low pH step. Finally, the eluted protein was neutralized and its concentration calculated using the asorbance at 280 nm and the molar extinction coefficient. The aggregate content of the antibody sample was analysed by analytical size exclusion chromatography using a Zorbax GF-250 column (Agilent Cat No PSMO 845006).

The results of this purification procedure are summarized in table 6

Table 6: Summary of purification results, SEQ ID NOs see Table 8

anti DR5 mAb	Titer [mg/ml]	Yield [mg/ml]	Monomer content [%]
3E6	47.3	32.9	100
4C3	43.6	21.2	100
5E11	38.2	29.9	100
5F6	40.4	38.0	100
6A8	41.9	28.1	100
6F8	64.4	45.1	99
7B12	75.1	44.6	100
7H9	54.7	38.5	99
18F11	77.6	54.4	100
18F12	66.6	33.4	99
18H6	54.6	48.5	100
19F10	61.1	36.7	100
20F2	65.9	41.0	99
21C3	51.4	40.6	97
212	53.6	27.1	99
23C7	49.8	27.2	100
24B7	38.5	28.9	100
1B12	179.3	78.1	100
2C5	116.2	48.7	98
2E5	144.3	67.7	100
2F11	146.2	67.3	100
4A6	148.0	83.2	100
4F5	110.5	48.7	100
4G9	128.1	80.9	100
6F6	161.3	86.3	100
6H4	128.1	65.8	100

8D2	136.6	65.8	100
17A7	136.1	62.7	100
17H5	155.7	77.1	100
18A4	138.9	67.1	100
18A6	103.9	54.7	100
18A12	118.1	63.3	100
18E6	90.8	44.3	100
18F6	99.3	36.0	100
19C12	192.6	70.6	92
19D6	144.9	81.7	97
19G7	117.0	63.1	100
20E3	116.4	51.7	100
20F1	75.5	40.7	100
21H3	127.1	45.1	98
22E6	130.9	61.2	98
22E9	137.5	60.2	100
23G10	127.9	43.5	100
6F9	96.8	48.2	100
6G7	114.7	59.3	100
17A8	102.1	60.0	100

This small scale transfection and production followed by purification via ProteinA beads yielded in reasonable amounts of pure antibodies with very low aggregate content. In figure 13 (a-e) the results of a standard DNA fragmentation ELISA assay for detection of apoptosis are summarized. Each antibody was tested in two different concentrations (1.0 µg/ml: black bars and 0.1 µg/ml: hatched bars with 1.0 and 0.1 µg/ml of anti-Fc antibody for cross-linking, respectively). The obtained data were normalized to the maximal activity of cross-linked Drozitumab (at 1.0 µg/ml) which was set to 100%. The majority of analyzed antibodies were able to induce concentration dependent induction of apoptosis in MDA-MB-231 cells after cross-linking. Seven of 46 binders (15.2 %) clearly showed higher activity as Drozitumab at both concentrations. Fifteen antibodies (32.6 %) were in the same activity range as Drozitumab and twenty of the tested molecules (43.5 %) did induce apoptosis upon cross-linking but to a much lower degree compared to Drozitumab. The remaining four binders (“20E3”, “19E6”, 1B12” and “19C12”, 8.7 %) were inactive and did not induce apoptosis, even after cross-linking via a secondary anti Fc antibody.

Example 7: Characterization of novel DR5 binders

Based on the results of apoptosis activity screen twelve of 46 evaluated DR5 antibodies were selected for additional, more detailed analysis and characterization. These antibodies were re-produced in transiently transfected HEK293 EBNA cells and purified using standard ProteinA

affinity columns followed by size exclusion chromatography as described. Yield and monomer / aggregate content were determined (table 7). The purified antibodies were characterized with respect to target specificity, species cross-reactivity and affinity (table 8). In addition thermal stability was analyzed by DLS and the apoptosis induction activity was compared in the presence or absence of a secondary cross-linking anti-human Fc antibody.

Table 7: Series of selected DR5 antibodies, SEQ ID NOs see Table 8

Antibody	SEQ ID NO. VH	SEQ ID NO. VL	Yield [mg/L]	Monomer [%]	Specificity	Cross-reactivity
18F11	94	95	33.00	100.0	specific for DR5 no binding to DR5, DcR1, DcR2 or OPG	Human and cynomolgous monkey DR5 no binding to murine DR5
18H6			8.40	71.2	as above	as above
18E6			31.50	100.0	as above	as above
6H4			30.30	100.0	as above	as above
5F6			42.18	100.0	as above	as above
20F2	106	107	30.00	100.0	as above	as above
4G9			42.90	100.0	as above	as above
22E9	100	101	39.96	100.0	as above	as above
21H3	102	103	32.35	100.0	as above	as above

4F5			36.63	100.0	as above	as above
5E11	7	8	31.68	100.0	as above	as above
24B7			19.73	100.0	as above	as above

Table 8: Characterization of novel DR5 binders

Antibody	SEQ ID NO. VH / VL	Affinity [nM]	Avidity [nM]	Aggregation temperature [°C]
18F11	94/95	504	1.1	64
18H6		555	2.7	n.d.
18E6		471	2.2	66
6H4		773	8.9	65
5F6		552	1.5	66
20F2	106/107	431	4.8	66
4G9		478	6.4	65
22E9	100/101	217	1.5	64
21H3	102/103	259	1.6	64
4F5		575	4.4	n.d.
5E11	7/8	162	1.1	65
24B7		300	2.3	65

All analyzed antibodies specifically recognize human DR5 and do not bind to the closest human homologs from the TNFR super family such as DR4, decoy receptors (DcR1 and DcR2) and osteoprotegerin (OPG). All DR5 antibodies are cross-reactive with human and cynomolgus DR5 but do not recognize the murine counterpart (which is not unexpected due to a sequence homology of only about 30%). While the affinities to human DR5 are in a quite wide range (from 162 to 773 nM) all measured avidities are in the low (one digit) nanomolar range. All tested DR5 binders reveal a high thermal stability with aggregation temperatures well above 60 °C as measured by Dynamic Light Scattering (DLS) experiments.

To determine functional activity of a series of selected DR5 binders, apoptosis induction was analyzed using a Cell Death Detection ELISA assay (Roche; #11 774 425 001) which specifically detects DNA fragmentation. Antibodies were used at a concentration of 1.0 and 0.1 µg/ml in the presence of the same concentration of secondary anti human Fc antibody for cross-linking. For comparison antibodies were used at 1.0 µg/ml in the absence of the secondary

antibody to evaluate if the activity of the selected DR5 binders depends on cross-linking or if they are already active on their own. The human breast cancer cell line MDA-MB-231 was used as the target cell line. In figure 14 the results of the apoptosis induction of different DR5 binders are summarized. After cross-linking all of the newly isolated DR5 antibodies induce apoptosis in the same range as the control antibody Drozitumab. However, unlike Drozitumab which already exhibits significant apoptosis induction activity without cross-linking, activity of the novel DR5 binders strictly depends on cross-linking via a secondary antibody. The apoptotic activity does not seem to be correlated to the affinity of the DR5 binder to its target since this was similar for antibodies with both the highest and the lowest affinity to human DR5.

Two additional assays were used to evaluate the activity of selected DR5 binders: Inhibition of proliferation upon treatment with cross-linked antibodies was measured with a CellTiter-Glo assay (Promega #TB288). In addition, induction of Caspase 8 was determined by a Caspase8-Glo assay (Promega #G8202). Figure 15 shows the maximal inhibition of proliferation at a concentration of 7 nM of DR5 binders cross-linked via secondary anti Fc antibody of three different tumor cell lines. The effect on the human colorectal adenocarcinoma cell line DLD-1 (black bars) was compared to the large cell lung cancer line HCI-H460 (hatched bars) and the human breast cancer cell line MDA-MB-231 (white bars). For all three cell lines significant inhibition of proliferation could be detected. While the inhibition of proliferation of DLD-1 at 7 nM seems to correlate with the affinity of the DR5 antibody this is not the case for NCI-H460 and only to a certain degree for MDA-MB-231.

Table 9: Calculated IC50 values for inhibition of proliferation using the CellTiter-Glo assay

Antibody	**SEQ ID NO. VH / VL**	DLD-1	NCI-H460	MDA-MB-231
5E11	7/8	0.50	3.91	> 7.00
22E9	100/101	0.56	4.00	> 7.00
21H3	102/103	1.57	6.20	> 7.00
24B7		1.86	5.00	> 7.00
20F2	106/107	2.01	6.53	> 7.00
4G9		4.30	> 7.00	> 7.00
18E6		4.62	6.89	> 7.00
5F6		6.39	> 7.00	> 7.00
18F11	94/95	> 7.00	> 7.00	> 7.00
4F5		> 7.00	> 7.00	> 7.00
6H4		> 7.00	> 7.00	> 7.00

Figure 16 summarizes the results of Caspase 8 activation upon treatment with anti Fc cross-linked DR5 antibodies. The maximal Caspase 8 activation in three different tumor cell lines (DLD-1, black bars; NCI-H460, hatched bars; and MDA-MB-231, white bars) is shown at a concentration of 7 nM. For all antibodies high level of Caspase 8 activation could be detected in all three cell lines. Induction of Caspase 8 was in the same range for the different cell lines but

the correlation of Caspase 8 induction level with the affinity of the antibodies was not as pronounced as seen in the CellTiter-Glo Assay for inhibition of proliferation.

Table 10: Calculated EC50 values for induction of Caspase 8 using the Caspase 8-Glo assay

Antibody	**SEQ ID NO. VH / VL**	DLD-1	NCI-H460	MDA-MB-231
5E11	7/8	0.13	0.03	0.14
22E9	100/101	0.15	0.05	0.24
21H3	102/103	0.20	0.09	0.31
24B7		0.21	0.09	0.26
20F2	106/107	0.31	0.13	0.33
4G9		0.48	0.38	1.04
18E6		0.33	0.13	0.35
5F6		0.76	0.28	1.00
18F11	94/95	0.36	0.37	0.43
4F5		0.49	0.16	0.89
6H4		1.84	0.70	0.99

Example 8: Epitope analysis

To characterize the kind and relative localization of the epitopes recognized by the newly isolated DR5 binders in more detail, Western / Dot Blot analysis and Biacore measurements have been performed with purified IgGs. Comparison of Western Blot vs. Dot Blot results would show if the antibodies recognize a linear or a conformational epitope while Biacore competition experiments would hint to different, identical or partially overlapping epitopes.

To differentiate between linear or conformational epitopes human DR5 was separated by SDS-PAGE, blotted onto a Nitrocellulose membrane, incubated with the different DR5 binders and detected with a secondary anti-huFc-HRP antibody (Sigma A0170). In parallel human DR5 was spotted onto a membrane, DR5 antibody was added and detected with the same secondary antibody. Binding of the antigen only in the Dot Blot experiment would hint to a conformational epitope since in this setting the antigen is analyzed in its natural three dimensional conformation whereas in the Western Blot experiment the antigen has been denatured and only linear epitopes should be accessible.

Table 11: Summary of results from Western / Dot Blot analysis

Anti DR5 mAb	**SEQ ID NO. VH / VL**	Signal in Western Blot	Signal in Dot Blot
4G9		+	+++
6H4		+	++

5F6		+++	+++
18F11	94/95	++	+++
18H6		++	+++
20F2	106/107	+	+++
18E6		+	+++
22E9	100/101	-	++
21H3	102/103	-	+++
4F5		-	++
5E11	7/8	-	+++
24B7		+++	+++

Based on these results it could be concluded that the majority of the analyzed antibodies recognize a conformational epitope on hu DR5 while only two antibodies (5F6, 24B7) clearly bind to the denatured antigen in Western Blot analysis indicating binding to a linear epitope. Strong binding in Dot Blot and weak binding in Western Blot also hint to a conformational epitope but this probably contains linear stretches that are recognized (18F11, 18H6).

Binding competition assays by Surface Plasmon Resonance (SPR, Biacore) were performed in two different settings. In the classical sandwich assay a first DR5 binder is immobilized on a chip followed by addition and binding of hu DR5. Then the second DR5 antibody is injected and analyzed for additional binding to DR5. In the tandem assay hu DR5 is immobilized on a chip followed by addition of a first DR5 binder. Then the second DR5 binder is injected and additional binding is analyzed. Figure 17 summarizes the results of these binding competition assays assays (comparison of four new DR5 antibodies with Drozitumab). From these competition assays it could be concluded that the clones 5E11, 22E9 and 174 (VH SEQ ID NO.:88, VL SEQ ID NO.:89, see e.g. Example 26) probably share a common epitope since once one of these has been bound to DR5 none of the two others could bind to the antigen. These three antibodies clearly recognize a different epitope as Drozitumab. The binding site of clone 422 (VH SEQ ID NO:82, VL SEQ ID NO.:85 see e.g. Example 26) might overlap with the epitope of 5E11, 22E9 and Drozitumab but definitely not with the one from clone 174. A clear answer to the question of the recognized epitope might come from co-crystallization experiments (DR5 + mAb) followed by structure analysis.

To evaluate if the DR5 antibodies recognize an epitope that is similar or overlaps with the DR5 ligand binding site a TRAIL competition assay in Biacore was set up. For that purpose recombinant hu TRAIL (Preprotech No 310-04) was immobilized on a CM5 chip. A preformed complex consisting of human DR5-Fc and the DR5 binder was used as analyte and binding to the immobilized TRAIL was determined. In figure 18 the results of the TRAIL competition experiment are shown. With the exception of clone 422 (dotted line in figure 18b) all newly isolated DR5 binders seem to bind to an epitope that at least overlaps with the TRAIL binding

site on DR5 since none of the tested complexes is able to bind to immobilized hu TRAIL (all solid lines). In contrast to that, DR5 alone (dashed line) or a complex of DR5 with a control antibody (short dashes) showed clear binding to the immobilized ligand.

Example 9: Non-ligand competing DR5 antibodies demonstrate increased in vitro apoptosis activity in the presence of TRAIL compared to ligand competing binders

During isolation and screening of new DR5 binders, antibodies were isolated which recognize different epitopes on human DR5. One possible criterion to classify the antibodies is to group them in ligand blocking and ligand non-blocking molecules. Among the selected binders one representative for each of these groups was chosen: clone 5E11 was identified to block TRAIL binding to DR5, while clone 422 is a ligand non-blocking antibody (figure 18).

To evaluate the impact of binding to an epitope that at least overlaps with TRAIL binding, these two candidates were analyzed for inhibition of proliferation of DLD-1 CRC target cells after cross-linking with secondary anti Fc antibodies in the presence or absence of human TRAIL. 4000 DLD-1 target cells per well were incubated over night at 37 °C before cross-linked antibodies were added: the antibodies 5E11, 422 and a control antibody that does not block TRAIL were used in six different concentrations (starting from 6.67 nM in 2.5 fold dilution steps down to 0.068 nM). Cross-linking was achieved by addition of equimolar concentration of anti Fc secondary antibody. TRAIL was added in concentrations of 10, 4, 1.6, 0.64, 0.256 and 0.102 nM to the respective antibody concentration. After 48 hrs incubation Cell TiterGlo reagent was added. The results of this growth inhibition assay are shown in figure 19. It was demonstrated that clone 422, the non-ligand blocking antibody exhibited significantly increased anti proliverative activity upon addition of human TRAIL compared to clone 5E11 which binds to a TRAIL binding site overlapping epitope. Addition of cross-linked 5E11 antibody had no additive effect on growth inhibition by TRAIL alone. In contrast, using cross-linked clone 422 in combination with human TRAIL showed a significant additive effect on activity.

Similar results were observed with a relevant control antibody known to not block TRAIL binding on DR5. If this effect observed in an *in vitro* assay can be translated into *in vivo* settings remains to be evaluated.

Example 10: Conversion in bispecific 2+2 format

In order to evaluate whether the novel DR5 binders can be used for the generation of bispecific antibodies for the targeted induction of apoptosis of tumor cells by hyper-cross-linking of DR5, a set of DR5 antibodies were converted into tetravalent bispecific molecules. These bispecific antibodies contain two binding moieties, each for DR5 and FAP (fibroblast activation protein). Different, selected DR5 antibodies were combined with the FAP antibody 28H1, a high affinity, human/ murine /cynomolgous monkey (hu/mu/cy) cross-reactive FAP binder isolated

and affinity matured by phage display. In the used 2+2 format the 28H1 CrossFab domain (VHCL) was fused to the C-terminus of the anti DR5 heavy chain using a $(G_4S)_4$ connector. Schematic structures of the 2+2 format are shown in figure 28). Below are exemplary chains of the bispecific antibodies in the 2+2 format.

Table 12a: Bispecific, tetravalent DR5 – FAP CrossMab molecules (all with 28H1 CrossFab domain (VHCL) fused to the C-terminus of the anti DR5 heavy chain using a $(G_4S)_4$ connector, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.:16)

DR5 Binder	SEQ ID NO VH /VL (DR5)	**Name**	**Description**
22E9	100/101	DR5 (22E9)-28H1 VHCL 2+2	28H1 CrossFab domain (VHCL) fused to the C-terminus of the anti DR5 (22E9) heavy chain using a $(G_4S)_4$ connector: VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chain (SEQ ID NO.:125) VL (DR5)-kappa light chain (SEQ ID NO.:126) VLCH1 (FAP) chain (SEQ ID NO.:124).
21H3	102/103	DR5 (21H3)-28H1 VHCL 2+2	28H1 CrossFab domain (VHCL) fused to the C-terminus of the anti DR5 (21H3) heavy chain using a $(G_4S)_4$ connector: VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chain (SEQ ID NO.:125) VL (DR5)-kappa light chain (SEQ ID NO.:128) VLCH1 (FAP) chain (SEQ ID NO.:124).
20F2	106/107	DR5 (20F2)-28H1 VHCL 2+2	28H1 CrossFab domain (VHCL) fused to the C-terminus of the anti DR5 (20F2) heavy chain using a $(G_4S)_4$ connector: VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chain (SEQ ID NO.:129) VL (DR5)-kappa light chain (SEQ ID NO.:130) VLCH1 (FAP) chain (SEQ ID NO.:124).

5E11	7/8	DR5(5E11)-28H1 VHCL 2+2	28H1 CrossFab domain (VHCL) fused to the C-terminus of the anti DR5 (5E11) heavy chain using a $(G_4S)_4$ connector: VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chain (SEQ ID NO.:131) VL (DR5)-kappa light chain (SEQ ID NO.:132) VLCH1 (FAP) chain (SEQ ID NO.:124).
5E11	7/8	DR5(5E11)-28H1 VHCL 2+2 P329GLALA	As above, and removal of C-term. Lysine and P329G/LALA mutation in Fc VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chain (SEQ ID NO.:134) VL (DR5)-kappa light chain (SEQ ID NO.:132) VLCH1 (FAP) chain (SEQ ID NO.:124).
18F11	94/95	DR5(18F11)-28H1 VHCL 2+2	28H1 CrossFab domain (VHCL) fused to the C-terminus of the anti DR5 (18F11) heavy chain using a $(G_4S)_4$ connector: VH $_{(DR5)}$-Fc part – VH $_{(FAP)}$ -CL chain (SEQ ID NO.:140) VL (DR5)-kappa light chain (SEQ ID NO.:141) VLCH1 (FAP) chain (SEQ ID NO.:124).

Table 12b: Production data of bispecific, tetravalent DR5 – FAP CrossMab molecules

DR5 binder	Production yield [mg/L]	Final monomer [%]	Apoptosis induction
5E11	20.3	96.7	+++
22E9	18.1	99.0	+++
21H3	7.6	96.3	+++
20F2	19.3	100.0	+++
18E6	25.1	100.0	++
5F6	18.9	99.4	+
18F11	22.3	96.7	+
6H4	18.5	100.0	++
18H6	17.5	99.6	---

All DR5 – FAP bispecific molecules were produced in transiently transfected HEK293 EBNA cells and were purified via Protein A and size exclusion chromatography. The obtained product yields were in a reasonable range (around 20 mg/L). The monomer content after the final purification step was above 96 % for all molecules.

Target binding analysis by surface plasmon resonance (Biacore) revealed that all selected bispecific antibodies in the 2+2 format were able to simultaneously bind to recombinant DR5 and FAP (human and murine) as depicted in figure 42.

To evaluate if the DR5-FAP bispecific molecules are able to induce apoptosis of the MDA-MB-231 target cell line, 96 well plates were coated with recombinant human FAP for cross-linking of DR5 on the target cells via the subsequently added bispecific antibodies. After addition of the target cells (MDA-MB 231) and incubation for 24 hrs apoptosis induction was determined by the standard DNA fragmentation ELISA assay. Figure 20 shows the comparison of apoptosis induction of seven bispecific antibodies containing newly isolated DR5 binders and the C-terminally fused 28H1 FAP CrossFab compared to Drozitumab at two different concentrations (7.0 nM and 0.7 nM). All molecules were tested in the presence and absence of FAP. As expected, at high concentration the Drozitumab based bispecific molecule induced apoptosis already in the absence of cross-linking FAP to a significant degree. In contrast, the DR5-FAP bispecific molecules using the new DR5 binders only exhibited apoptosis induction activity in the presence of FAP coated on the plates indicating that this activity is dependent on the cross-linking via recombinant FAP.

To determine if the bispecific molecules also could be efficiently cross-linked via FAP expressed on a different cell line than DR5 and thereby induce apoptosis, a so-called bystander co-culture assay was set up. Figure 21 shows the results of apoptosis induction in a tumor cell line (MDA-MB-231) and FAP expressing fibroblast (GM05389) co-culture experiment with three different concentrations of bispecific molecules (7.0, 0.7 and 0.07 nM). The three bispecific constructs containing the DR5 binding moieties from 5E11, 22E9 and 20F2, respectively fused to the 28H1 FAP CrossFab show a comparable degree of induction of apoptosis as it also was observed with the corresponding, anti Fc cross-linked IgG molecules. In contrast to that the molecule containing 6H4 as the DR5 binding part exhibited reduced activity compared to cross-linked IgG. One of the tested molecules (18H6 – 28H1) was completely inactive at all concentrations (in contrast to the cross-linked IgG), indicating that not all DR5 binders are suitable for generation of bispecific molecules to hyper-cross-link DR5 on tumor cells. Therefore a careful evaluation of epitope and bispecific activity is necessary to choose the right DR5 antibody for the approach of targeted induction of apoptosis. In this regard DR5 binders which display a rather low affinity to DR5 and a high avidity in the 2+2 bispecific format are particularly advantageous. The low affinity for DR5 prevents binding of the bispecific

antibodies to normal cells and hence increases the selectivity for inducing apoptosis in tumor cells in a FAP dependent manner. One binder having these characteristics is for example the DR5 binder 5E11.

As described, one of the disadvantages of Drozitumab, which limits its use in bispecific formats, is the fact that this antibody does not allow for N-terminal fusion of a second binding moiety. This configuration leads to the blocking of Drozitumab's N-terminus which inhibits proper binding to DR5 and thereby induction of apoptosis is prevented. To test if the newly isolated DR5 antibodies can be used in this kind of format, Fc-fusion molecules were generated, in which the Fabs of selected DR5 binders were fused C-terminally to a human Fc region via a $(G_4S)_4$ connector. These molecules were transiently produced in HEK293 EBNA cells, purified via ProteinA beads and tested in an apoptosis induction assay. In figure 22 a) the results of the DNA fragmentation assay in MDA-MB-231 cells with these Fc-DR5 fusion molecules after cross-linking with secondary anti Fc IgG are summarized. All tested molecules are able to induce apoptosis of the target cell line, indicating that the chosen DR5 binders are not N-terminally blocked which opens a wider range of formats that can be used with these binders.

Example 11: Characterization of thermal stability of 2+2 bispecific format containing phage display derived DR5 binder

Thermal stability of phage display derived DR5 binder converted into the 2+2 bispecific format was measured using an Optim1000 system (Avacta Group plc) as the change in scattered light intensity. In a micro cuvette array, 9 µL of the samples in 20 mM Histidine, 140 mM NaCl, pH 6.0 at a concentration of 1 mg/mL were heated from 25 °C to 90 °C at a rate of 0.1 °C/min. Scattered light intensity (266 nm laser) was recorded every 0.4 °C and processed with the software IgorPro, Version 6.23 (Avacta Group plc). The aggregation onset temperature is defined as the temperature at which the scattering intensity starts to increase.

Table 13: Aggregation onset temperatures of bispecific antibodies (2+2 format) containing phage display derived DR5 binders as a measure for thermal stability of the bispecific constructs

TheraPS Name Bispec Antibody	Alias Bispec Antibody	SEQ ID NOs	Optim1000 Tagg (°C)
DR5TAA-0030	5E11-28H1	131,132, 124	64
DR5TAA-0037	22E9_28H1	124,125,126	63
DR5TAA-0038	21H3_28H1	127,128,124	63
DR5TAA-0039	20F2_28H1	129,130,124	64

All tested DR5 antibodies reveal a high thermal stability with aggregation temperatures well above 60 °C also when cloned into 2+2 bispecific constucts.

Example 12: DR5 – FAP bispecific antibodies are able to induce apoptosis on different target cells

Nine DR5 – FAP bispecific antibodies in the 2+2 format comprising newly isolated DR5 binders (four isolated by phage display, five derived from rabbit immunization as depicted in examples 22 to 25) fused to the FAP 28H1 CrossFab moiety were compared in a side-by-side experiment for induction of apoptosis on two different cell lines (MDA-MB-231 and G401) in a co-culture assay with GM05389 human FAP^+ fibroblasts. The bispecific antibodies were tested over a concentration range from 0.0007 – 7 nM. The results of the DNA fragmentation assay are summarized in figure 23 (MDA-MB-231) and figure 24 (G401). All bispecific antibodies tested demonstrated good apoptosis induction activity on both cell lines. According to the results obtained with the MDA-MB-231 cells the antibodies could be divided into two groups. The one obtained from rabbit immunization showed the maximum of apoptosis induction at a concentration of 0.7 nM and with further increasing antibody concentration the activity decreased slightly. In contrast, the phage display derived binders in the bispecific 2+2 format did not show the decline in activity at high concentrations but stayed constant or even more increased up to the highest concentration. In the experiment with G401 cell in co-culture with GM05389 fibroblasts for both sets of bispecific antibodies the maximum of apoptosis induction was reached already at a concentration of 0.07 nM and then stayed constant. In this setting all molecules performed similarly in terms of apoptosis induction levels.

Example 13: Comparison of different bispecific formats

It has been demonstrated that DR5-FAP bispecific molecules in the 2+2 CrossFab format produce well in a very good quality and are able to mediate concentration-dependent specific induction of apoptosis in a two cell line co-culture setting. The degree of apoptosis induction of these bispecific molecules is in the same range as observed with the corresponding DR5 binders that were hyper-cross-linked via a secondary anti Fc antibody. To evaluate additional, different DR5-FAP bispecific molecules for their apoptosis induction capacity, the following constructs have been generated in which DR5 and FAP binding entities are combined in different formats and with different valences as shown in figure 25.

Table 14: Description of alternative DR5-FAP bispecific formats

	Description	Valency	DR5	FAP

1	Fusion of FAP binder as CrossFab (VHCL) to the C-terminus of DR5 heavy chain (knob). B24_E11A_001 DR5(5E11)-28H1Fc knob VHCL 2+1 (SEQ ID NO. 142) DR5(5E11) Fc hole (SEQ ID NO. 143) FAP (28H1) VLCH1 (SEQ ID NO. 124) DR5(5E11) LC (SEQ ID NO. 132) Comment: Knob-into-hole; (G4S)4 connector, Figure 25a	2+1	5E11	28H1
2	FAP CrossFab (VHCL) on Fc-hole. DR5 Fabs (head-to-tail) fused to Fc-knob. B24_E11E_001 DR5(5E11)_Fc knob Fab-Fab Head-to-tail 2+1 (SEQ ID NO. 145) FAP (28H1)_Fc holeVHCL (SEQ ID NO. 146) FAP (28H1) VLCH1 (SEQ ID NO. 124) DR5(5E11) LC (SEQ ID NO. 132) Comment: Knob-into-hole;(G4S)2 connector, Figure 25b	2+1	5E11	28H1
3	FAP CrossFab (VHCL) fused to the C-terminus of DR5 Fc-hole. DR5 Fab fused to C-terminus of DR5 Fc-knob. B24_E11A_002 DR5(5E11)-28H1 Fc knobVHCL 3+1 (SEQ ID NO. 143) DR5(5E11)-DR5(5E11) Fc hole 3+1(SEQ ID NO. 144) FAP (28H1) VLCH1 (SEQ ID NO. 124) DR5(5E11) LC (SEQ ID NO. 132) Comment: Knob-into-hole; (G4S)4 connector, Figure 25c	3+1	5E11	28H1
4	Fusion of FAP binder as CrossFab (VHCL) to the C-terminus of DR5 heavy chain (knob). B16_E11A_001 DR5(18F11)-28H1Fc knobVHCL 2+1 (SEQ ID NO. 147) DR5(18F11) Fc hole (SEQ ID NO. 148) FAP (28H1) VLCH1 (SEQ ID NO. 124) DR5(18F11) LC (SEQ ID NO. 141) Comment: Knob-into-hole; (G4S)4 connector, Figure 25a	2+1	18F11	28H1
5	FAP CrossFab (VHCL) fused to the C-terminus of DR5 Fc-hole. DR5 fused to C-terminus of DR5 Fc-knob. B16_E11A_002 DR5(18F11)-28H1VHCLFc knob 3+1 (SEQ ID NO. 149) DR5(18F11)-DR5(18F11) Fc hole (SEQ ID NO. 150) FAP (28H1) VLCH1 (SEQ ID NO. 124) DR5(18F11) LC (SEQ ID NO. 141) Comment: Knob-into-hole; (G4S)4 connector, Figure 25c	3+1	18F11	28H1

All molecules were transiently produced in HEK293 EBNA cells and were purified according to standard Protein A and size exclusion chromatography protocols. Side product profile and quality of the molecules were analyzed by SDS-PAGE, SEC and Caliper analysis.

Table 15: Summary of production results of additional bispecific DR5-FAP molecules

	Construct	Description	Yield [mg/ml]	Aggregates [%]	Monomer [%]	Low molecular weight species [%]
1	B24_E11E_001	5E11_28H1; 2+1 C-terminal on Fc-knob	5.42	1.55	95.2	3.25
2	B24_E11A_001	5E11_28H1; head-to-tail on Fc-knob	5.99	0.74	98.08	0.74
3	B24_E11A_002	5E11_28H1; 3+1 C-terminal on Fc-knob	5.39	1.80	98.20	0.00
4	B16_E11A_001	5E11_28H1; 2+1 C-terminal on Fc-knob	18.34	1.50	98.50	0.00
5	B16_E11A_002	5E11_28H1; 3+1 C-terminal on Fc-knob	3.32	0.90	99.10	0.00

As summarized in table 15 these additional formats seem to be more difficult to be produced and purified compared to the 2+2 formats. Except one (B16_E11A_001), all show lower expression yields as the analogous 2+2 molecules. However, the aggregate contents were rather low (< 2%) but in most cases also lower molecular weight species could be detected which might be due to purification or degradation of the molecules.

Apoptosis induction activity on MDA-MB-231 was tested in the fibroblast co-culture assay (DNA fragmentation) at different concentrations (7.0; 0.7 and 0.07 nM). Figure 26 shows the results of apoptosis activity of three different formats (2+2; 2+1 and 3+1) compared to bispecific Drozitumab in the 2+2 format. In this setting the 5E11-28H1 bispecific molecule in the 2+2 format overall showed similar activity compared to the Drozitumab control. At the lowest concentration, the 5E11-28H1 bispecific molecule in the 2+2 format had displayed even higher activity compared to the Drozitumab control. In contrast, the 2+1 and the 3+1 formats seemed to be less active compared to both 2+2 formats (Drozitumab and 5E11). If the same formats are analyzed with a different DR5 binder (18F11) all three molecules show reduced apoptosis induction activity as compared to the Drozitumab bispecific antibody. However, with the 18F11

bispecific molecule the maximal apoptosis induction occurred at lower concentrations (0.07 nM) whereas the Drozitumab based molecule exhibits higher activity with increasing concentration.

In a second set of molecules an additional 2+1 format was evaluated for the 5E11-28H1 bispecific antibody in which two DR5 binding moieties are fused head-to-tail to an Fc-knob part while the FAP targeting Fab is combined with an Fc-hole counterpart. All four formats were compared over a broad range of concentrations (ranging from 7.0 to 0.0007 nM) in a DNA fragmentation assay for apoptosis induction of MDA-MB-231 in co-culture with GM05389 fibroblasts (figure 27). Both 2+1 molecules had the highest apoptosis activity at a concentration of 0.7 nM while the 3+1 molecule showed maximal induction of apoptosis already at a 10 fold lower concentration. However, none of the new formats seemed to be superior over the conventional 2+2 format.

Example 14: Test of functional activity of a 1+1 bispecific format in a co-culture assay system

Besides the different format and valency variants described in example 12, another construct has been generated in a 1+1 format (as depicted in fig. 25 D) with the DR5 binder 0011 (= clone 174) in the uncrossed Fab and the FAP binder 4B9 in the crossed Fab (SEQ ID NOs. 280-282). Functional activity of the 1+1 format was compared to a 2+2 format (as depicted in fig. 28 A, containing 0011 as DR5 binder and 28H1 as FAP binder; SEQ ID NOs 287-289) and to the chimeric IgG of 0011 (SEQ ID NOs 90-93) with or without secondary crosslinking.

Apoptosis induction was measured by Cell Death Detection ELISA (CDDE, Roche #11 774 425 001) upon treatment of cells with anti-DR5 and anti-DR5-FAP bispecific antibodies in the presence or absence of a cross-linking secondary antibody in a co-culture system consisting of tumor cells (MDA-MB-231) and fibroblasts (GM05389).

Day 1: Preparation of cells. The adherent MDA-MB-231 cell line (human breast adenocarcinoma) was grown in DMEM medium (PAN) supplemented with 10% fetal calf serum (Gibco) and 2mM L-glutamine (PAA), and normally split twice per week 1:20. The GM05389 fibroblasts were grown in MEM + Earle's medium (Gibco) supplemented with 15% fetal calf serum (Gibco), 1x NEAS (PAN) and 2mM L-glutamine (PAA), and normally split twice per week 1:3.
For co-culture assays fibroblast were seeded on day 1 at a density of $1x10^4$ cells/100µl/well in 96-well plates and incubated overnight at 37°C, 5% CO2. Tumor cells and antibodies were added on day 2.

For monoculture assays cell lines were seeded on different plates at the same density, incubated overnight and treated with antibodies the next day.

Day 2: Induction of apoptosis. Medium was aspirated and antibodies (anti-DR5 IgGs or anti-DR5-FAP bispecific antibodies) were added to the cells at different final concentrations (see figures) alone or together with the cross-linking antibody (goat anti-human IgG, Sigma #I2136) in a 1:1 ratio in 100µl medium.

For co-culture assays tumor cells were immediately added ($1x10^4$ cells/100µl/well) on the fibroblasts after the antibodies to reach a final volume of 100µl.

Cultures were incubated at 37°C for 24 hs.

Day 3: Cell Death Detection Elisa (CDDE). The immunoassay was performed according to the manufacturer's instructions (Roche) with slight changes. Briefly, cells were lysed with 100µl/well 2x-lyse buffer for 15 minutes at RT. A master mix consisting of anti-histone and anti-DNA antibodies was prepared according to the manufacturer's instructions and mixed with 1:4 diluted lysates on 96-well streptavidin-coated flat-bottomed microtiter plates (Roche). After a 2-hour incubation at RT, wells were washed, ABTS substrate added and incubated at RT until color development sufficient for photometric analysis (10-30 min). Absorbance was read at 405nm with a Tecan Spectra Rainbow Reader.

Results are shown in Figures 46 and 47.

While the monospecific anti-DR5 antibodies (both chimeric 0011 and Drozitumab) did not induce significant apoptosis by their own, hyperclustering of the DR5 molecules on the cell surface with a secondary antibody led to cell death at concentrations around 0.7 mM. However, best apoptosis induction was achieved when anti-DR5-FAP bispecific antibodies (in either format 1+1 or 2+2) were added to the co-culture leading to DR5 hyperclustering on the tumor cells via the second moiety which binds to FAP present on the surface of fibroblasts (figure 46).

While the monospecific anti-DR5 antibodies induced apoptosis of MDA-MB-231 cells upon cross-linking, bispecific molecules against DR5 and FAP were only functional in the presence of DR5-expressing tumor cells and FAP-expressing tumor associated fibroblasts. In addition, none of the molecules showed any effect on the viability of fibroblasts. (Relative apoptosis as compared to internal assay positive control, see figure 47).

This activity data demonstrates that FAP specific apoptosis induction can also be achieved with a DR5-FAP bispecific antibody in a 1+1 format with one valency for each target in a comparable efficacy as with a 2+2 format.

Example 15: The 2+2 bispecific format as a generic platform technology

The bispecific DR5-FAP CrossFab molecules in the 2+2 format in which the 28H1 VHCL is fused to the C-terminus of the 5E11 heavy chain has been proven to be a very good format. For different DR5 binders it has demonstrated to be producible at reasonable product titers, it is stable, exhibits only low amounts of missing or wrongly paired light chains and reproducibly shows good activity. Nevertheless, to extend the format platform, additional bispecific 5E11-28H1 molecules have been generated which differ in the kind and location of the crossing point. The five additional formats evaluated and the parental molecule are depicted in figure 28. Four of the five additional molecules do not contain a crossed 28H1 Fab anymore but a standard Fab domain fused to the C-terminus of the DR5 (5E11) heavy chain (either VHCH1 or VLCL which is fused by a $(G_4S)_4$ connector).

Table 16: Description of additional DR5-FAP CrossFab molecules

Fig. 28 scetch	B	C	D	E	F
Kind of Crossing	N-terminal Fab	N-terminal VHVL	N-terminal CH1CL	C-terminal Fab	C-terminal VHVL
5E11 IgG	VLCL – Fc	VLCH1 – Fc	VHCL – Fc	VHCH1 – Fc	VHCH1 – Fc
28G1 Fab (fusion to heavy chain C-terminus)	VHCH1	VHCH1	VHCH1	VLCL	VLCH1

Table 17: Exemplary sequences of additional DR5-FAP CrossFab molecules

VLCL DR5 (5E11)_28H1 2+2 (molecule B in Fig 28)	Anti FAP 28H1 Fab (VHCH1) fused to the C-terminus of the DR5 (5E11) heavy chain by a (G4S)4 connector with entire Fab crossed (VLCL-Fc) VLCL (DR5) – Fc – VHCH1 (FAP) (Seq ID NO. 274) VHCH1 (DR5) (SEQ ID NO. 275) VLCL (FAP 28H1) (SEQ ID NO. 137)
VHVL DR5(5E11)-28H1 2+2 (molecule C in Fig 28)	Anti FAP 28H1 Fab (VHCH1) fused to the C-terminus of the DR5 (5E11) heavy chain by a (G4S)4 connector with crossing in Fab (VLCH1-Fc) VL (DR5)-CH1- Fc part-VH(FAP)-CH1 chain (SEQ ID NO. 135) VH(DR5) –CL (SEQ ID NO.:136)

	VL (FAP) –kappa light chain (SEQ ID NO.:137).
CH1CL DR5(5E11)-28H1 2+2 (molecule D in Fig 28)	Anti FAP 28H1 Fab (VHCH1) fused to the C-terminus of the DR5 (5E11) heavy chain by a (G4S)4 connector with crossing in Fab (VHCL1-Fc) VH (DR5) CL- Fc- -VH(FAP) -CH1 chain (SEQ ID NO.:138) VL(DR5) –CH1 chains (SEQ ID NO.:139) VL (FAP) –kappa light chains (SEQ ID NO.:137)
DR5 (5E11)-28H1 (VLCL) 2+2 (molecule E in Fig 28)	Anti FAP (28H1) Fab (VLCL) fused by a (G4S)4 connector to C-terminus of DR5 (5E11) Fc VH (DR5)-CH1-Fc-VL (FAP)-CL (SEQ ID NO. 276) VL (DR5)-CL (SEQ ID NO. 132) VH (FAP)-CH1 (SEQ ID NO. 277)
DR5 (5E11)-28H1 (VLCH1) 2+2 (molecule F in Fig 28)	Anti FAP (28H1) CrossFab (VLCH1) fused by a (G4S)4 connector to the C-terminus of DR5 (5E11) Fc VH (DR5)-CH1-Fc-VL (FAP)-CL (SEQ ID NO.278) VL (DR5)-CL (SEQ ID NO. 132) VH (FAP)-CL (SEQ ID NO. 279)

All molecules were transiently produced in 200 ml scale in HEK293 EBNA cells, purified by standard ProteinA and size exclusion chromatography and finally analyzed and characterized in comparison with the original format (C-terminal CH1CL crossing of 28H1 fused to 5E11 heavy chain, (molecule A, fig 28)).

As summarized in table 16 the five molecules can be divided into two groups: one contains the two formats in which the entire Fab's are crossed (B + E). These two molecules gave very low product titers and even after purification they still contained high amounts of aggregate. Therefore these two molecules were not further evaluated. The other three (crossing of 5E11 VHVL or CH1CL and crossing of the C-terminal 28H1 as VHVL) exhibited much better product quality with respect to yield and aggregate content. The molecules C, and D also were tested for target binding on MDA-MB-231 cells by FACS in comparison to molecule A as shown in figure 29. Furthermore functionaly activity in terms of apoptosis induction, simultaneous and independent target binding, stability and aggregation tendency as well as side product patterns of molecules C, D, and F were compared to format A (Examples 22 to 25)

Example 16: Test of functional activity of 2+2 bispecific format variants in a coculture assay system

The four different 2+2 format variants (format A, C, D, F of fig 28) were compared for their functional activity to induce apoptosis as measured by Cell Death Detection ELISA (CDDE, Roche #11 774 425 001) upon treatment of cells with the constructs in a co-culture system consisting of tumor cells (MDA-MB-231) and fibroblasts (GM05389).

Day 1: Preparation of cells. The adherent MDA-MB-231 cell line (human breast adenocarcinoma) was grown in DMEM medium (PAN) supplemented with 10% fetal calf serum (Gibco) and 2mM L-glutamine (PAA), and normally split twice per week 1:20. The GM05389 fibroblasts were grown in MEM + Earle's medium (Gibco) supplemented with 15% fetal calf serum (Gibco), 1x NEAS (PAN) and 2mM L-glutamine (PAA), and normally split twice per week 1:3.

For co-culture assays fibroblast and tumor cells were seeded on the same day each at a density of $1x10^4$ cells/100µl/well in 96-well plates and incubated overnight at 37°C, 5% CO_2. For monoculture assays cell lines were seeded on different plates at the same density.

Day 2: Induction of apoptosis. Medium was aspirated and bispecific antibodies were added to the cells at different final concentrations (see figures) in 100µl medium.

Cultures were incubated at 37°C for 24 hs.

Day 3: Cell Death Detection Elisa (CDDE). The immunoassay was performed according to the manufacturer's instructions (Roche) with slight changes. Briefly, cells were lysed with 100µl/well 2x-lyse buffer for 15 minutes at RT. A master mix consisting of anti-histone and anti-DNA antibodies was prepared according to the manufacturer's instructions and mixed with 1:4 diluted lysates on 96-well streptavidin-coated flat-bottomed microtiter plates (Roche). After a 2-hour incubation at RT, wells were washed, ABTS substrate added and incubated at RT until color development sufficient for photometric analysis (10-30 min). Absorbance was read at 405nm with a Tecan Spectra Rainbow Reader.

Results are shown in Figure 30. Induction of apoptosis of 4 different CrossMab variants in co- (a) and mono-culture (b, c) settings as detected by DNA fragmentation. All 4 different variants induce apoptosis in tumor cells in the co-culture setting in a comparable dose-dependent manner. In mono-culture settings no apoptosis is induced neither in MDA-MB231 tumor cell line nor in GM05389 fibroblast cell line, pointing out the specificity and FAP-dependency of apoptosis

induction of all 4 variants. This functional activity data demonstrates that the bispecific 2+2 format can be used in different configurations with respect to type and site of crossing.

Example 17: Test of independent and simultaneous target binding of 2+2 bispecific format variants

The four different 2+2 format variants (format A, C, D, F of fig 28) were compared additionally for their ability to bind both targets independently and simultaneously by SPR assays.

Assessment of independent DR5- and FAP-binding to different Crossmab Variants

Around 3000 resonance units (RU) of the capturing system (5 µg/ml anti human IgG (Fc); GE Healthcare, BR-1008-39) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) pH 7.4. The temperature of the flow cell was set to 25 °C and of the sample block to 12 °C. Before capturing, the flow cell was primed with running buffer twice.

The bispecific antibody was captured by injecting a 5µg/ml solution for 60 sec at a flow of 5 µl/min. Independent binding of each ligand to the bispecific antibody was analysed by determining the active binding capacity for each ligand, either added sequentially or simultaneously (flow of 10 µl/min):

1. Injection of human DR5 with a concentration of 5µg/ml for 180 sec (identifies the single binding of the antigen).

2. Injection of human FAP with a concentration of 5µg/ml for 180 sec (identifies single binding of the antigen).

3. Injection of human DR5 with a concentration of 5µg/ml and of human FAP with a concentration of 5µg/ml for 180 sec (identifies the binding of DR5 and of FAP at the same time).

The surface was regenerated by 60 sec washing with a 3m MgCl2 solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from an anti human IgG surface.

The bispecific antibody is able to bind both antigens mutual independently if the resulting final signal of the approach 3 equals the sum of the individual final signals of the approaches 1 and 2.

Table 18: Quantitative Assessment of the independent DR5- and FAP-binding of 4 different Crossmab variants

	SEQ ID NO	DR5	FAP	expected signal DR5 + FAP	measured signal Mix: DR5 + FAP	ratio expected measured
		[RU max]	[RU max]	[RU max]	[RU max]	[%]
DR5TAA-0057 format A	134,132,124	37,1	40,8	77,9	76,7	98
DR5TAA-0077 format C	135,136,137	33,0	41,9	74,9	73,1	98
DR5TAA-0078 format D	138,139,137	27,1	38,4	65,5	63,6	97
DR5TAA-0081 format F	278,132,279	26.7	7.2	34	33	97

All 4 different Crossmab variants are able to bind DR5 & FAP mutually independent.

Assessment of simultaneous DR5- and FAP-binding to the Crossmab

First, around 600 resonance units (RU) of DR5 (20µg/ml) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween 20) pH 7.4. Flow cell was set to 25 °C and sample block to 12 °C and primed with running buffer twice. Second, 10 µg/ml solution of the bispecific antibody was injected for 30 sec at a flow of 30 µl/min. Third, hFAP (10µg/ml) was injected for 30 sec at a flow of 30 µl/min. The binding response of hFAP depends from the amount of the bispecific antibody bound to hDR5 and shows simultaneous binding. The surface was regenerated by 70 sec washing with a Glycine pH2 solution (GE Healthcare BR-1003-55) at a flow rate of 30 µl/min. Simultaneous binding is shown by an additional specific binding signal of hFAP to the previous DR5 bound Crossmab.

Assessment of the simultaneous DR5- and FAP-binding of four Crossmab formats (DR5TAA-0057, DR5TAA-0078, DR5TAA-0077 and DR5TAA-0081) showed that all 4 different Crossmab variants are able to bind FAP and DR5 simultaneously.

Example 18: Test of thermal stability and aggregation tendency of 2+2 bispecific format variants

The four different 2+2 format variants (format A, C, D, F of fig 28) were furthermore analyzed for their thermal stability and for their tendency to form aggregates.

Thermal Stability

Aggregation onset temperature: Samples were prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffin oil. The hydrodynamic radius is measured repeatedly by dynamic light scattering while the samples are heated with a rate of 0.05 °C/min from 25 °C to 80 °C. The aggregation onset temperature is defined as the temperature at which the hydrodynamic radius starts to increase.

Aggregation tendency

Samples were dialyzed into formulation buffer (20 mM His/HisCl, 240 mM Trehalose, pH 6.0) and adjusted to a concentration of 1 mg/mL. After sterile filtration over 0.22 µm centrifugal filter devices (Millipore), samples were stored for 2 weeks at 40°C, while a control sample was maintained at -80°C. Aggregate formation was monitored by SE-HPLC using a TSK3000 SWXL column (Tosoh) and reported as the difference between the 40°C and the -80°C sample.

Table 19: Assessment of the thermal stability and aggregation tendency of 4 different Crossmab formats.

	DR5TAA-0057 (SEQ ID NOs 134,132,124) Format A	**DR5TAA-0077 (SEQ ID NOs 135, 136, 137) Format C**	**DR5TAA-0078 (SEQ ID NOs 138, 139, 137) Format D**	**DR5TAA-0081(SEQ ID NOs 278, 132, 279) Format F**
Stability by DLS [Tagg, °C]	64	61	60	61
Aggregate formation during storage @ 40 °C in formulation buffer [% increase]	0.4	0.3	2.2	0.5

Thermal stability was slightly reduced in the formats C, D and F as compared to the parental format A but with 61 °C and 60 °C still in a good range.
Aggregation tendency was very low for formats A, C and F and slightly increased for format D.

Example 19: Evaluation of side-product profile of 2+2 bispecific format variants by mass spectrometry

Finally, the four different 2+2 format variants (format A, C, D, F of fig 28) were furthermore analyzed for their side-product profile by mass spectrometry.
The deglycosylated total mass of the different constructs was determined and confirmed via electrospray ionization mass spectrometry (ESI-MS). Moreover potential side products such as LC overrepresentation were detected and relatively quantified. Briefly, 100 µg purified antibodies at a protein concentration of up to 3 mg/ml were deglycosylated with 14 U N-Glycosidase F (Roche) in 100 mM NaH_2PO4/Na_2HPO4, pH 7 at 37°C for 2 h and subsequently desalted via HPLC on a Sephadex G25 column (GE Healthcare). The deglycosylated total mass was determined via ESI-MS on a maXis UHR-TOF (Bruker) MS system equipped with a TriVersa NanoMate (Advion) source.
The different DR5-FAP bispecific antibody constructs were analyzed by mass spectrometry in their deglycosylated form to evaluate the product identity and integrity. The identity could be confirmed for all evaluated constructs. Moreover the different constructs showed several side products for example with overrepresentation of one LC type or with loss of one or two LCs. All constructs had qualitatively and quantitatively similar byproducts profiles.

All results from examples 13 and 30-33 celarly show that the bispecific 2+2 format can be used in different configurations with respect to type and site of crossing. This makes the 2+2 CrossFab format a broadly applicable platform technology.

Example 20: Drozitumab-FAP bispecific molecules exhibit superior in vivo efficacy over untargeted Drozitumab

It has been demonstrated in *in vitro* activity assays that bispecific Drozitumab-FAP exhibit superior apoptosis induction activity compared to Drozitumab alone which is not cross-linked. To evaluate if this also translates into *in vivo* efficacy in relevant mouse tumor models different xenograft models have been set up. One problem with the standard mouse tumor models is that the FAP expression usually is very low and does not reflect the human situation at all. To

establish models that more closely resemble the FAP expression in human tumor stroma, engineered murine fibroblasts (3T3 cells) that recombinantly express mouse FAP were co-injected with the respective tumor cell lines (colorectal carcinoma cell line DLD-1 in nude mice and breast cancer line MDA-MB-231 in SCID mice). For this purpose 3T3 fibroblasts were stably transfected with a plasmid that carries an expression cassette for the full length murine FAP gene under control of the MPSV promoter. For selection of stable clones the vector further carries an additional expression cassette for a puromycin acetyltransferase which confers resistance to puromycin. Several clones expressing different levels of murine FAP as judged by FACS binding experiments have been selected. One selected mu FAP-3T3 cell line was co-injected with tumor cell line DLD-1 leading to significantly enhanced tumor growth as compared to DLD-1 cells injected without mu FAP expressing 3T3 cells. Also, IHC analysis using human / mouse cross-reactive anti FAP antibodies has demonstrated high levels of FAP expression in the tumor surrounding stroma for both cell lines, as expected. Therefore this co-grafted subcutaneous xenograft model was used to assess the *in vivo* efficacy of bispecific Drozitumab-FAP molecule compared to the original untargeted Drozitumab antibody. $2x10^6$ tumor cells were co-implanted with 20 % of 3T3 fibroblast expressing murine FAP. Twelve (DLD-1) and ten days (MDA-MB-231) after tumor cell implantation therapy was started. The animals were injected (i.v.) either buffer, Drozitumab (10 mg / kg) or Drozitumab-FAP (10 mg / kg). To compensate for the molecular weight difference of Drozitumab vs. bispecific Drozitumab (Drozitumab is only 60 % of the molecular mass of the bispecific molecule) the latter was administered twice weekly while Drozitumab was only given once a week. In figure 31 the increase of mean tumor volumes in mm^3 over time are summarized. Figure 31 A shows *in vivo* efficacy of Drozitumab-FAP compared to Drozitumab and the buffer control in the DLD-1 / mu FAP-3T3 co-injection model. While Drozitumab only demonstrated a moderate anti-tumor efficacy, resulting in a tumor growth inhibition (TGI) of 36 % compared to the control, the bispecific molecule exhibited a tumor growth inhibition of 99 % at the end of the study (day 33). In the MDA-MB-231 / mu FAP-3T3 co-injection model this difference is even more pronounced since in this model Drozitumab alone did not show any efficacy better than the buffer control whereas the Drozitumab-FAP bispecific antibody exhibited a tumor growth inhibition of 77 % at the end of the study at day 32 (figure 31 B). This result might indicate that the MDA-MB-231 cell line is more resistant to apoptosis induction as the DLD-1 cell line.

Example 21: Evaluation of anti-tumor activity of DR5-FAP bispecific molecules using newly isolated DR5 binders in combination with 28H1 FAP CrossFab.

In vivo efficacy of three different DR5 binders (5E11, 174 and 422) in bispecific format fused to the anti FAP antibody 28H1 as CrossFab was compared side-by-side in the DLD-1 and MDA-MB-231 xenograft models each co-injected with 3T3 fibroblast expressing murine FAP. A

fourth molecule consisting of 5E11-28H1 CrossFab with mutations in the Fc region that completely abolish binding to Fcγ receptors (while affinity to FcRn is unchanged) was included. For this purpose large scale transient transfections and productions were conducted to generate sufficient material in HEK cells.

Table 20: Production of bispecific antibodies for in vivo experiments. All materials exhibited acceptable endotoxin content of < 0.23 EU / mg.

	Sample	Fc region	Titer [mg/L]	Yield [mg]	Aggregate [%]
1	DR5(5E11)-FAP(28H1)VHCL 2+2	wt	60	75	< 2
2	DR5(5E11)-FAP(28H1)VHCL 2+2	PG_LALA*	80	100	< 2
3	DR5(174)-FAP(28H1)VHCL 2+2	wt	n.d.	65	< 1
4	DR5(422)-FAP(28H1)VHCL 2+2	wt	n.d.	96	< 1

* L234A; L235A; P329G

All molecules were produced at good yields with excellent quality with respect to aggregate and endotoxin content. Binding to the relevant antigens was analyzed by different methods (SPR and FRET) as summarized in table 21.

Table 21 a: Affinities / Avidities of DR5-FAP bispecific molecules to human and cynomolgus antigens

	Sample	**Fc region**	**Affinity [nM]***		**Avidity [nM]***		**Avidity [nM] +**	
			hu DR5	cy DR5	hu DR5	cy DR5	hu DR5	cy DR5
1	DR5(5E11)-FAP(28H1)VHCL 2+2	wt	146.0	9.6	0.16	0.29	1.2	0.4

2	DR5(5E11) - FAP(28H1) VHCL 2+2	PG_LALA	147.0	11.9	0.14	0.26	1.9	0.6
3	DR5(174)-FAP(28H1) VHCL 2+2	wt	9.8	20.2	0.10	3.72	1.1	0.3
4	DR5(422)-FAP(28H1) VHCL 2+2	wt	5.1	2.0	0.08	n.d.	0.8	0.7

* Biacore measurements + TagLite

Before *in vivo* experiments were initiated the materials were first tested for *in vitro* apoptosis induction activity. Figure 32 summarizes the results of a Cell Death Detection ELISA in which four different DR5-FAP bispecific molecules were compared at concentrations of 7.0, 0.7 and 0.07 nM. The assay was set up as a co-culture assay in which DLD-1 cells were used as targeted cells and 3T3 or recombinant 3T3 cells expressing murine FAP served as the effector cells for cross-linking. In the DLD-1 – 3T3 co-culture experiment hardly any induction of apoptosis was detectable in DLD-1 cells whereas in the setting with the FAP expressing 3T3 cells a 10 fold increase in apoptosis induction was observed indicating that this activity is due to the cross-linking via FAP on the surface of the recombinant 3T3 cells. A similar experiment was performed in which the same bispecific molecules, target and effector cells were used to determine cell viability upon treatment with the bispecific agonistic DR5-FAP antibodies. The results of this experiment are summarized in figure 33. Here, a significant reduction of cell viability of DLD-1 cells only was observed in the presence of FAP expressing 3T3 cells while with unmodified 3T3 cells (which do not express murine FAP) no reduction of viability was seen.

All four bispecific DR5-FAP molecules were used for evaluation of *in vivo* efficacy in two different tumor models, both co-injected with 20 % of murine FAP expressing 3T3 fibroblasts. The results of these *in vivo* efficacy experiments are shown in figure 34. After engraftment of the tumor and fibroblast cells treatment started with 10 mg / kg administered twice weekly intravenously (i.v.). Definitely, all four molecules were able to control tumor growth in both models as demonstrated by significant tumor growth inhibition (TGI) compared to the vehicle control. The absolute percentage of tumor growth inhibition at the end of the study was in a similar range for all four molecules. The following results were obtained for the DLD-1 and MDA-MB-231 model, respectively: 5E11_28H1 (wt Fc): 75 % / 95 %; 5E11_28H1 (PGLALA): 83 % / 99 %; clone 174: 66 % / 87 % and clone 422: 73 % / 89 %. All molecules were slightly more potent in the MDA-MB-231 model than in the DLD-1 experiment.

Table 21 b): Summary of characteristics of preferred DR5-FAP bispecific antibody

	DR5	FAP
Clone Name	5E11 (VH: SEQ ID NO.: 7 VL: SEQ ID NO.:8)	28H1 (VH: SEQ ID NO.: 15 VL: SEQ ID NO.: 16)
Affinity human [nM]	165 (IgG)	2.6 (IgG)
Affinity Cyno [nM]	1.02 (IgG)	3.7 (IgG)
Avidity Human [nM]	0.06 (IgG)	0.25 (IgG)
Avidity Cyno [nM]	0.06 (IgG)	0.06 (IgG)
Binding Mode	agonistic (only upon crosslinking) TRAIL competitive, conformational epitope	No interference with signaling/protease function, conformational epitope
Specificity	No binding to huDR4, DcR1/2, OPG	No binding to hu DPP-IV (CD26, closest FAP homologue)
Species Cross-Reactivity	Human, cyno	Human, cyno, murine

Examples 22 to 25: Generation and characterization of new DR5 binding moieties by immunization

Besides selection of new DR5 antibodies with improved properties from a phage display library (as described above) new DR5 antibodies were generated also by immunization of rabbits (examples 22-25) followed by intensive characterization (examples 26-28).

Example 22: Immunization of rabbits

One set of rabbits was immunized with 400 µg of recombinant human DR5 (monomeric Fc fusion), emulsified with complete Freund's adjuvant, at day 0 by intradermal application, and with 200 µg each of DR5-huFc, emulsified with complete Freund's adjuvant, at days 7, 14, 35, 63 and 91, by alternating intramuscular and subcutaneous applications. Blood (10% of estimated total blood volume) was taken at days 21, 41, 69 and 97. Serum was prepared, which was used for titer determination by ELISA (see below), and peripheral mononuclear cells were isolated, which were used as a source of antigen-specific B cells in the B cell cloning process (Example 17).

Another set of rabbits was immunized genetically, using a plasmid expression vector coding for human DR5 lacking the intracellular death domain, by intradermal application of 400 µg vector DNA, followed by Electroporation (5 square pulses of 750 V/cm, duration 10 ms, interval 1 s). Rabbits received 6 consecutive immunizations at days 0, 14, 28, 49, 77 and 105.

Blood (10% of estimated total blood volume) was taken at days 35, 56, 84 and 112. Serum was prepared, which was used for titer determination by ELISA (see below), and peripheral mononuclear cells were isolated, which were used as a source of antigen-specific B cells in the B cell cloning process (Example 23).

Determination of serum titers

Human DR5 (monomeric Fc fusion), was immobilized on a 96-well NUNC Maxisorp plate at 0.3125 µg/ml, 100 µl/well, in PBS, followed by blocking of the plate with 2% Crotein C in PBS, 200 µl/well; application of serial dilutions of antisera, in duplicates, in 0.5% Crotein C in PBS, 100 µl/well; detection with HRP-conjugated donkey anti-rabbit IgG antibody (Jackson Immunoresearch) diluted 1:16 000 in 0.5% Crotein C in PBS, 100 µl/well. For all steps, plates were incubated for 1 h at 37° C. Between all steps, plates were washed 3 times with 0.05% Tween 20 in PBS. Signal was developed by addition of BM Blue POD Substrate soluble (Roche), 100 µl/well; and stopped by addition of 1 M HCl, 100 µl/well. Absorbance was read out at 450 nm, against 690 nm as reference. Titer was defined as dilution of antisera resulting in half-maximal signal.

Example 23: B-Cell Cloning from Rabbits

Isolation of rabbit peripheral blood mononuclear cells (PBMC)

Three rabbits (described in the Example "Immunization of rabbits") were used as a source of blood. EDTA containing whole blood was diluted twofold with 1x PBS (PAA, Pasching, Austria) before density centrifugation using lympholyte mammal (Cedarlane Laboratories, Burlington, Ontario, Canada) according to the specifications of the manufacturer. The PBMCs were washed twice with 1x PBS.

EL-4 B5 medium

RPMI 1640 (Pan Biotech, Aidenbach, Germany) supplemented with 10% FCS (Hyclone, Logan, UT, USA), 2mM Glutamin, 1% penicillin/streptomycin solution (PAA, Pasching, Austria), 2mM sodium pyruvate, 10mM HEPES (PAN Biotech, Aidenbach, Germany) and 0,05 mM b-mercaptoethanole (Gibco, Paisley, Scotland)

Depletion of macrophages/monocytes

Sterile 6-well plates (cell culture grade) were used to deplete macrophages and monocytes through unspecific adhesion. Each well was filled at maximum with 4 ml medium and up to $6x10^6$ PBMCs from the immunized rabbit and allowed to bind for 1 h at 37 °C in the incubator. The cells in the supernatant (peripheral blood lymphocytes (PBLs)) were used for the antigen panning step.

Coating of plates

For panning on protein sterile streptavidin coated 6-well plates (Microcoat, Bernried, Germany) were coated with 2 µg/ml biotinylated recombinant human DR5 (monomeric Fc fusion) in PBS for 3 h at room temperature. For panning on human surface DR5-positive cells G401 cells were seeded in sterile cell culture 6-well plates and cultivated to generate a confluent cell monolayer. Prior to the panning these 6-well plates were washed with sterile PBS three times.

Enrichment of B cells on the human DR5 protein

6-well tissue culture plates coated with human DR5 protein or covered with human DR5-positive G401 cells were seeded with up to $6x10^6$ PBLs per 4 ml medium and allowed to bind for 1 h at 37 °C in the incubator. After the enrichment step on the DR5 antigen non-adherent cells were removed by carefully washing the wells 1-2 times with 1x PBS. The remaining sticky cells were detached by trypsin for 10 min at 37 °C in the incubator. Trypsination was stopped with EL-4 B5 medium. The cells were kept on ice until the immune fluorescence staining.

Immune fluorescence staining and Flow Cytometry

The anti-IgG FITC (AbD Serotec, Düsseldorf, Germany) was used for single cell sorting. For surface staining, cells from the depletion and enrichment step were incubated with the anti-IgG FITC antibody in PBS and incubated for 45 min in the dark at 4°C. After staining the PBMCs were washed two fold with ice cold PBS. Finally the PBMCs were resuspended in ice cold PBS and immediately subjected to the FACS analyses. Propidium iodide in a concentration of 5 µg/ml (BD Pharmingen, San Diego, CA, USA) was added prior to the FACS analyses to discriminate between dead and live cells. A Becton Dickinson FACSAria equipped with a computer and the FACSDiva software (BD Biosciences, USA) were used for single cell sort.

B-cell cultivation

The cultivation of the rabbit B cells was prepared by a method similar to that described by Zubler et al. (1985). Briefly, single sorted rabbit B cells were incubated in 96-well plates with 200 µl/well EL-4 B5 medium containing Pansorbin Cells (1:100000) (Calbiochem (Merck), Darmstadt, Deutschland), 5% rabbit thymocyte supernatant (charge TSN-M13 (10242), MicroCoat, Bernried, Germany) and gamma-irradiated murine EL-4-B5 thymoma cells (2,5 × 104/well) for 7 days at 37 °C in an atmosphere of 5% CO2 in the incubator. The supernatants of the B-cell cultivation were removed for screening and the remaining cells were harvested immediately and were frozen at – 80 °C in 100 µl RLT buffer (Qiagen, Hilden, Germany).

Example 24: B-cell PCR and Recombinant Expression

Isolation of ribonucleic acid (RNA)

The cells from which the RNA had to be isolated were at first pelleted by centrifugation. The cell pellet was lysed by the addition of 100 µl RLT-buffer with 10 µl/ml beta-mercaptoethanol. The cells were resuspended by multiple mixing with a pipette and transferred to a multi well plate. The plate was shortly centrifugated at 200 x g and frozen at -20 °C. The isolation of the RNA was performed with the NucleoSpin® 96 RNA kit (Macherey & Nagel) according to the manufacturer's instructions.

Reverse transcription polymerase chain reaction

The reverse transcription was carried out with SuperScript III First-Strand Synthesis SuperMix (Invitrogen) according to the manufacturer's instructions.

Polymerase chain reaction

The polymerase chain reaction was carried out with AccuPrime Pfx SuperMix (Invitrogen) according to the manufacturer's instructions. Light chain and heavy chain variable regions were amplified in separate reactions. PCR-primers were used with 25 bp overlaps to target antibody expression vectors. PCR-products were purified by NucleoSpin® 96 Extract II kit (Macherey & Nagel).

Sequencing and SLIC Cloning

The PCR products were sequenced to determine the DNA-sequences of the variable regions of heavy and light chains. The PCR-products were cloned into expression vectors by the so called SLIC-cloning method, which is described by Haun, R.S., et al., in BioTechniques 13 (1992) pp. 515-518 and Li, M.Z., et al., in Nature Methods 4 (2007) pp. 251-256. The plasmids for the antibody expression were linearized by restriction anzyme digestion. The linearized plasmids were purified by preparative agarose electrophoresis and extracted from the gel (Qiaquick Gel Extraction Kit / Qiagen). The purified plasmids were added to a PCR-protocol using overlapping primers (bay 25 bp) for the PCR-product to be cloned. Both the vector and insert were treated with T4 DNA polymerase (Roche Applied Sciences) in the absence of dNTPs to generate overhangs, then vector and insert were incubated with RecA (New England Biolabs) protein and ATP to promote recombination. Products were transformed into E.coli. Plasmid DNAs for light chain and heavy chains were isolated and each couple was combined for transient transfections.

Transient Transfection for Antibody Expression in HEK293 cells

HEK293 cells (Invitrogen) were grown in F17-media (Gibco) to 1 x 10e6 cells/ml. 2 x 10e6 HEK293 cells were transfected with 1 µg HC + LC plasmids suspended in 293-free (Novagen) and OptiMEM® (Gibco). After 7 days incubation supernatants were harvested, purified via Protein A and analyzed.

Example 25: Screening of DR5 antibodies derived from immunization

B-cell culture supernatants were screened by multiple parallel ELISA-based binding assays in 384 well microtiter plates. Antibodies binding to the DR5-expressing cells G401 and to biotinylated recombinant human DR5 (monomeric Fc fusion) and cynomolgus DR5 (dimeric Fc fusion), but not to human DR4 (TNFRSF10A; R&D Systems Cat.No. 347-DR-100) and human IgG1, were selected as primary hits. In a secondary screening, micropurified antibodies recombinantly expressed in HEK cells were tested again for binding to human and cynomolgus DR5 and additionally for absence of binding to human DR4, human DcR1 (inhouse), human Dc R2 (in house) or human Osteoprotegerin (OPG; R&D Systems Cat.No. 805-05-100). Furthermore antibodies were tested in a functional apoptosis assay (Cell Death Detection Elisa) on G401 cells in the presence or absence of a cross-linking anti-human Fc antibody. Only those antibodies able to induce apoptosis upon Fc-mediated cross-linking (but not in its absence) were selected for further characterization and development.

Sequences of antibodies selected after secondary screening and cloned in expression plasmids by the SLIC cloning procedure were verified by subcloning and re-sequencing of the variable light and variable heavy chains.

These subcloned and sequence-verified expression plasmids were then used for larger scale transient transfections of HEK293F cells followed by Protein A purification allowing further more intensified characterization steps.

Example 26: Characterization of binding properties of DR5 antibodies derived from immunization

Selected DR5 antibodies from rabbit immunization were characterized for their binding properties, species cross-reactivity and specificity by binding ELISA and SPR analysis.

Binding of monoclonal antibodies to TRAIL binding receptors (Immunoassay)

Antigen binding immunoassays were performed at room temperature on 384 well streptavidin coated microtiter plates (MicroCoat Biotechnologie GmbH) with PBS buffer supplemented with 0.05% Tween®-20 and 0.5 % BSA (Roche Diagnostics GmbH). 125 ng/ml biotinylated human DR5 (monomeric Fc fusion) protein (inhouse) or 63 ng/ml biotinylated hFc cynomolgus DR5 protein (inhouse) or 63 ng/ml biotinylated DcR2 (TNFRSF10D) protein (inhouse) were added to the wells containing a mixture of 1:3000 diluted anti-rabbit Fc–HRP conjugate (GE Healthcare) and 1:50 diluted B-cell supernatants. After 90 min incubation the plate was washed 6 times with PBST (phosphate buffered saline with 0.2%Tween®-20) and developed with BM blue® HRP substrate solution (BM blue®: 3,3´-5,5´-Tetramethylbenzidine,

Roche Diagnostics GmbH) for 30 minutes at RT. Absorbance was measured at 370 nm. The blank value was defined without addition of supernatant.

For negative selection against the hFc tag of the immunogen an immunoassay with a mixture of biotinylated anti-human IgG (Fab specific) from Jackson ImmunoResearch (Cat. No. 109-066-006), human IgG1 (inhouse) and anti-rabbit Fc–HRP conjugate was used and processed as described. For testing binding to related Trail binding receptors like human DR4 (TNFRSF10A; R&D Systems Cat.No. 347-DR-100), human Osteoprotegerin (OPG; R&D Systems Cat.No. 805-05-100) and human DcR 1 (TNFRSF10C; R&D Systems Cat.No. 630-TR-100) an immunoassay was established by capturing the respective protein – hFc chimera with anti-human Fc antibody (Jackson ImmunoResearch, Cat. No. 109-006-098) on a MaxiSorp 384 well microtiter plate (Sigma-Aldrich, Nunc).

Table 22: Binding of DR5 antibodies (rabbit IgG) derived by immunization to human DR5 (monomeric Fc Fusion) and cynomolgus DR5 as detected by biochemical ELISA (EC50 [ng/ml]). No significant binding to mouse DR5, human DR4, DcR1, DcR2, and Osteoprotegerin was detected (data not shown)

DR5 Antibody Clone Name	DR5 Antibody Clone Alias	EC50 human DR5 [ng/ml]	EC50 cynomolgus DR5 [ng/ml]	SEQ ID NO VH / VL
DR5TAA-0005	039	2,5	3,7	41/46
DR5TAA-0006	058	2,0	1,6	51/55
DR5TAA-0010	481	2,8	8,7	60/64
DR5TAA-0013	298	2,1	2,1	68/71
DR5TAA-0019	461	3,5	2,2	74/78
DR5TAA-0016	422	4,0	2,7	82/85
DR5TAA-0011	174	2,0	4,3	88/89

Selected DR5 antibodies from rabbit immunization show good and comparable binding properties to human and cynomolgus DR5 in ELISA while they do not recognize murine DR5. All selected antibodies are highly specific for DR5 as they did not give significant signals in binding ELISA to human DR4, DcR1, DcR2, and Osteoprotegerin.

DR5 kinetic affinity

Around 3000-5000 resonance units (RU) of the capturing system (10 µg/ml goat anti rabbit; ordering code JIR111-005-046; Jackson Immuno Research) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) pH 7.4. The flow cell was set to 25 °C - and the sample block set to 12 °C - and primed with running buffer twice.

The DR5 antibodies were captured by injecting a 1 µg/ml solution for 30 sec at a flow of 10 µl/min. Association was measured by injection of recombinant human DR5 (monomeric His-Avi fusion protein, in house) in various concentrations in solution for 120 sec at a flow of 30 µl/min starting with 100 nM down to 0.41 nM in 1:3 dilutions. The dissociation phase was monitored for up to 300 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 sec washing with a 100mM H3PO4 (phosphoric acid) solution at a flow rate of 30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a blank surface. Buffer injections are also subtracted (= double referencing). For calculation of KD and other kinetic parameters the Langmuir 1:1 model was used.

Table 23: Kinetic affinities of DR5 antibodies (rabbit IgG) derived by immunization

DR5 Antibody Clone Name	DR5 Antibody Clone Alias	ka [nM]	kd [nM]	KD [nM]	SEQ ID NO VH / VL
DR5TAA-0005	039	1,60E+06	2,14E-02	1,33E-08	41/46
DR5TAA-0006	058	3,12E+05	1,85E-03	5,92E-09	51/55
DR5TAA-0010	481	1,11E+06	1,77E-02	1,59E-08	60/64
DR5TAA-0011	174	4,55E+05	1,93E-03	4,24E-09	88/89
DR5TAA-0013	298	4,07E+05	3,71E-03	9,12E-09	68/71
DR5TAA-0016	422	3,46E+05	5,02E-04	1,45E-09	82/85
DR5TAA-0019	461	9,00E+05	6,07E-04	6,75E-10	74/78

Example 27: Functional characterization of DR5 antibodies derived from immunization

DNA fragmentation and cell viability

DR5 antibodies were functionally characterized by evaluating apoptosis and cell viability as measured by Cell Death Detection ELISA (CDDE, Roche #11 774 425 001) and Cell Titer Glo (CTG, Promega #G7573), respectively, upon treatment with anti-DR5 antibodies in the presence or absence of a cross-linking antibody.

Preparation of cells: the adherent MDA-MB-231 cell line (human breast adenocarcinoma) was grown in DMEM medium (PAN) supplemented with 10% fetal calf serum (Gibco) and 2mM L-glutamine (PAA), and normally split twice per week 1:10. For the assay, cells were washed with PBS, detached from the flask with Accutase (PAA), seeded in 96-well flat-bottomed microtiter plates (Costar) at a density of $1x10^4$ cells/well (CDDE) or $0.25x10^4$ cells/well (CTG) in 50µl and incubated overnight at 37°C, 5% CO2.

Induction of apoptosis by rabbit anti-DR5 antibodies: samples (rabbit anti-DR5 IgGs, DR5-TAA-#) were added to the cells at different concentrations alone or together with the cross-linking antibody (goat anti-rabbit IgG, Jackson Immunoresearch) in a 1:1 ratio in 50µl in PBS, to

induce crosslinking of the DR5 receptors leading to apoptosis. Cells were incubated at 37°C for 24 hs (CDDE) or 48hs (CTG).

A) Cell Death Detection Elisa (CDDE): the immunoassay was performed according to the manufacturer's instructions (Roche). Briefly, supernatants were carefully aspirated and cells lysed with 200 µl/well lyse buffer for 30 minutes at RT. A master mix consisting of anti-histone and anti-DNA antibodies was prepared according to the manufacturer's instructions and mixed with the 1:4-diluted lysates on 96-well streptavidin-coated flat-bottomed microtiter plates (Roche). After a 2-hour incubation at RT, wells were washed, ABTS substrate added and incubated at RT until color development sufficient for photometric analysis (10-30 min). Absorbance was read at 405nm with a Tecan Spectra Rainbow Reader.

Figure 35 shows the results of this experiment: The generated rabbit anti-DR5 antibodies were able to induce apoptosis of MDA-MB231 cells with different potencies but always in a dose-dependent fashion and only after Fc-mediated cross-linking of the DR5 molecules. In the absence of an anti-rabbit Fc-specific secondary antibody, no significant cell death was detected.

B) Cell Titer Glo (CTG): the immunoassay was performed according to the manufacturer's instructions (Promega). Briefly, cells were first lysed in the buffer containing the luminescence substrate (100µl). After a 10-minute incubation period on a shaker luminescence was measured with the TECAN Infinite Plus reader.

Figure 36 shows the results of this experiment: Cell viability is diminished by anti-DR5 antibodies upon receptor hyperclustering in a dose-dependent manner. The generated rabbit anti-DR5 antibodies were able to decrease the viability of MDA-MB231 cells with varying potencies but always in a dose-dependent fashion and only after Fc-mediated cross-linking of the DR5 molecules. In the absence of an anti-rabbit Fc-specific secondary antibody, the cell viability was not affected at any antibody concentration.

Cell viability & Caspase 8 activation

Caspase8-Glo Caspase 8 activation assays (Promega cat#G8202) and CellTiter-Glo cell viability assays (Promega cat#TB288) were carried out according to the manufacture's instructions.

For Caspase 8 activation assays, 10,000 cancer cells were seeded in 75 µl per well in opaque white 96 well plates (BD Falcon cat#BD353296) and incubated at 37°C with 5% CO2 overnight. Then anti-DR5 antibodies were added together with anti-rabbitFc antibodies (equal molar concentration of DR5 and rabbitFc antibodies in 25µl) in 6 serial dilutions. Antibodies were incubated on the cells at 37°C with 5% CO2 for 3 hours. Then 100 µl of caspase 8 substrate in lysis buffer was added to each well and mixed well. After incubation of another 30 minutes at 37°C luminescence signal was read in a Spectra Max M5 plate.

For Cell Viability assays, 4,000 cancer cells were seeded per well in black/clear bottom 96 well plates (BD Falcon cat#BD353220) and incubated at 37°C with 5% CO2 overnight. Then

anti-DR5 antibodies were added together with anti-rabbitFc antibodies (equal molar concentration of DR5 and rabbitFc antibodies in 25µl) in 6 serial dilutions. Antibodies were incubated on the cells at 37°C with 5% CO2 for 48 hours. Then 100 µl CellTiter-Glo reagent was added to each well and mixed well. After incubation of another 10 minutes at room temperature luminescence signal was read in a Spectra Max M5 plate reader.

Figure 37 shows the analysis of inhibition of cell proliferation (Cell TiterGlo Assay) of three different human tumor cells (DLD-1, NCI H460 and MDA-MB-231) upon treatment with different, cross-linked DR5 antibodies at a concentration of 7 nM. Figure 38 shows apoptosis induction measured by Caspase 8 activation in three human tumor cell lines (DLD-1, NCI H460 and MDA-MB-231) after treatment with cross-linked DR5 antibodies at a concentration of 7 nM. All tested DR5 antibodies induce high caspase 8 activation in all tested tumor cell lines. DR5 antibodies were able to decrease the viability of all tested cell lines with varying potencies.

Example 28: Evaluation of chemical stability of DR5 antibodies derived by phage display and immunization

Generation of stressed DR5 antibody samples

To test the chemical stability of DR5 antibodies, stressed samples were generated and functionally characterized with regard to DR5 binding. High-pH stress induces – among others – deamidation of reactive Asn hotspots, whereas at pH 6.0 e.g. succinimide formation from reactive Asn and Asp residues may be induced. For high-pH stress, samples were transferred in 20 mM Na-phosphate, pH 8.0 and incubated for 5 days at 40°C. For low-pH stress, samples were transferred into in 20 mM His/HisCl, 140 mM NaCl, pH 6.0 and incubated for 3 weeks at 40°C. A control sample was kept at -80°C.

Determination of the relative active concentration of stressed DR5 antibody samples

Around 5000 resonance units (RU) of the capturing system (20 µg/ml goat anti rabbit; ordering code JIR111-005-046; Jackson Immuno Research) were coupled on a CM5 chip (GE Healthcare BR-1005-30) at pH 5.0 by using an amine coupling kit supplied by the GE Healthcare. The sample and system buffer was PBS-T (10 mM phosphate buffered saline including 0.05% Tween20) pH 7.4. The temperature of the flow cell was set to 25 °C and of the sample block to 12 °C. Before capturing, the flow cell was primed with running buffer twice.

The bispecific antibody was captured by injecting a 50 nM solution for 60 sec at a flow of 10 µl/min. Association was measured by injection of human DR5 in solution for 90 sec at a flow of 30 µl/min at a concentration of 200 nM. The dissociation phase was monitored for up to 90 sec and triggered by switching from the sample solution to running buffer. The surface was regenerated by 60 sec washing with a 0.85% H3PO4 (phosphoric acid) solution at a flow rate of

30 µl/min. Bulk refractive index differences were corrected by subtracting the response obtained from a blank surface.

The relative active concentration of the stressed antibody is the ratio calculated from the capture level and binding level (RU binding divided by RU capture, in comparison to the unstressed reference sample).

Figure 39 shows an exemplary response curve used for the determination of the relative active concentration of stressed DR5 antibody samples. Figure 40 and 41 show relative active concentrations of original and stressed DR5 antibodies derived from immunization and from phage display. Besides antibodies DR5TAA-0013 and DR5TAA-0010 that showed slightly deminished relative active concentrations after stress at pH6 and pH8, respectively, all other DR5 antibodies did not show any relevant reduction of relative active concentrazion after pH stress.

Example 29: Functional characterization of DR5-binder derived by immunization and phage display in 2+2 bispecific format in co-culture assays

In order to evaluate whether the novel DR5 binders derived by immunization can be used for the generation of bispecific antibodies for the targeted induction of apoptosis of tumor cells by hyper-cross-linking of DR5, a set of DR5 antibodies were converted into 2+2 bispecific molecules (as depicted in fig 18 A) in combination with the FAP antibody 28H1. Bispecific constructs were tested for their apoptosis inducing activity in a co-culture assay.

DLD-1 or H460 tumor cells (10,000 cells/well) are seeded to 96-well plates in co-culture with 3T3 cells or with 3T3 cells transfected to express murine FAP 2,500 cells/well) in a total volume of 150µl to allow for triplicate samples for each treatment. After 24h, cells are treated with 50µl antibodies (4X concentration) for 24h (untreated control: 50µl medium).

Cell Death Detection ELISA: (Roche Applied Science Cat. No. 11774 425 001): Procedure is followed exactly as stated in manufacturer's protocol. Vmax values are extrapolated from the measurement of abs at 405nm (minus reference wavelength value at 490nm) every minute for 10 minutes. An average of triplicate background values (lysis buffer alone) are subtracted from all samples. Data is expressed as fold increase over apoptosis in untreated samples.

Figure 43 shows induction of apoptosis in DLD-1 and H460 tumor cell lines by 2+2 bispecific constructs in co-culture assays as detected by DNA fragmentation. While a bispecific construct containing Drozitumab as DR5-binding component already induces apoptosis in the absence of FAP, all constructs containing new DR5 binders derived by immunization only induce apoptosis in the presence of FAP. Constructs with newly developed DR5 binders, such as

0011-28H1 and 0016-28H1, are able to induce apoptosis to a higher extent especially at low concentrations as compared to the Drozitumab containing bispecific construct.

Example 30: Characterization of cellular binding of DR5-binder derived by immunization and phage display in 2+2 bispecific format

Cells were stained ($5x10^4$/50 µl) with 24 µg/ml of each DR5-FAP construct or Drozitumab in staining buffer (PBS + 5% FCS) for 1 h on ice. After washing twice the secondary antibody goat anti-human IgG-AF488 (Invitrogen #A11013) was added at 10 µg/ml and cells were again incubated 1 h on ice protected from direct light. After two more washing steps cells were measured at FACS Canto.

All tested constructs show binding to MDA-MB-231 with varying intensities (see Figure 44).

Example 31: Humanization of the VH and VL domains of DR5 antibodies derived by immunization

The rabbit DR5-binding antibody DR5TAA-0011 was humanized using frameworks identical to human germline sequences. The human germline sequences hVH_3_64 (GenBank accession No. M99682) and hVH3_16 (GenBank accession No P01767) were the 2 acceptors for the VH humanized variants and the human germline sequences hVK1_93 (Accession No. P04431) and hVK1_5 (GeneBank accession No. P01602) were the acceptors for VL humanization. Eight humanized DR5 antibodies comprising a heavy chain variable region construct selected from SEQ ID NOs. 23 and 26, and a light chain variable region construct selected from SEQ ID NOs 24, 29, 30, 31, and 32 were obtained and further characterized (Examples 27 to 29).

Table 24: Humanized DR5 binders derived by immunization

Variant	HC variant	SEQ ID NO.	LC variant	SEQ ID NO.
DR5TAA-0066	VH7	23	VL3	30
DR5TAA-0067	VH7	23	VL15	24
DR5TAA-0068	VH17	26	VL10	31
DR5TAA-0071	VH17	26	VL15	24
DR5TAA-0072	VH17	26	VL2	32
DR5TAA-0073	VH17	26	VL3	30
DR5TAA-0074	VH7	23	VL10	31
DR5TAA-0075	VH7	23	VL11	29

Example 32: Functional characterization of humanized DR5-binder derived by immunization

Humanized DR5 antibodies were functionally characterized by evaluating apoptosis as measured by Cell Death Detection ELISA (CDDE, Roche #11 774 425 001) upon treatment with humanized anti-DR5 antibodies in the presence or absence of a cross-linking antibody.

Preparation of cells: the adherent MDA-MB-231 cell line (human breast adenocarcinoma) was grown in DMEM medium (PAN) supplemented with 10% fetal calf serum (Gibco) and 2mM L-glutamine (PAA), and normally split twice per week 1:10. For the assay, cells were washed with PBS, detached from the flask with Accutase (PAA), seeded in 96-well flat-bottomed microtiter plates (Costar) at a density of $1x10^4$ cells/well (CDDE) in 50µl and incubated overnight at 37°C, 5% CO_2.

Induction of apoptosis by humanized anti-DR5 antibodies: samples (humanized anti-DR5 IgGs, DR5-TAA-#) were added to the cells at different concentrations alone or together with the cross-linking antibody (goat anti-human IgG, Sigma #I2136) in a 1:1 ratio in 50µl in PBS, to induce crosslinking of the DR5 receptors leading to apoptosis. Cells were incubated at 37°C for 24 hs (CDDE).

Cell Death Detection Elisa (CDDE): the immunoassay was performed according to the manufacturer's instructions (Roche). Briefly, supernatants were carefully aspirated and cells lysed with 200µl/well lyse buffer for 30 minutes at RT. A master mix consisting of anti-histone and anti-DNA antibodies was prepared according to the manufacturer's instructions and mixed with the 1:4-diluted lysates on 96-well streptavidin-coated flat-bottomed microtiter plates (Roche). After a 2-hour incubation at RT, wells were washed, ABTS substrate added and incubated at RT until color development sufficient for photometric analysis (10-30 min). Absorbance was read at 405nm with a Tecan Spectra Rainbow Reader. Apoptosis signals were normalized to the apoptosis of the chimeric anti-DR5 antibody at a concentration of 2µg/ml.

Results are shown in Figure 45: **A** Humanized Variants of DR5TAA-0011 (DR5TAA-0066 – DR5TAA-0075, black lines) induce apoptosis upon crosslinking with secondary antibody in a dose-dependent manner. Several humanized variants, such as DR5TAA-0067, DR5TAA-0071, DR5TAA-0074 and DR5TAA-0075, are able to induce apoptosis in a similar manner concerning maximum of induction and dose-dependency as compared to the chimeric variant (DR5TAA-0052, grey lines). **B** Humanized Variants of DR5TAA-0011 (DR5TAA-0066 – DR5TAA-0075) induce no apoptosis if not crosslinked by a secondary antibody. Thus, also the humanized

variants are suited to be used in bispecific formats to specifically induce apoptosis only in the presence of FAP.

The Fabs of selected humanized variants were were fused C-terminally to a human Fc region via a $(G_4S)_4$ connector. These molecules were transiently produced in HEK293 EBNA cells, purified via ProteinA beads and tested in an apoptosis induction assay. In figure 22 b) the results of the DNA fragmentation assay in MDA-MB-231 cells with these Fc-DR5 fusion molecules after cross-linking with secondary anti Fc IgG are summarized. All tested molecules are able to induce apoptosis of the target cell line, indicating that the chosen DR5 binders are not N-terminally blocked which opens a wider range of formats that can be used with these binders.

Example 33: Characterization of binding affinities of humanized DR5-binder derived by immunization

Humanized DR5-binder were characterization for their binding affinities by SPR analysis.
BIAcore characterization: A BIAcore 3000 instrument (GE Healthcare) was used with a CM5 sensor mounted into the system. The sensor was preconditioned by a 1 min injection at 100 µl/min of 0.1 % SDS, 50 mM NaOH, 10 mM HCl and 100 mM H3PO4. System buffer was 10 mM HEPES pH 7.4, 150 mM NaCl, 0.05% TWEEN 20. The sample buffer was the system buffer supplemented with 1 mg/mL carboxymethyldextran (Sigma). An anti-human antibody capture system was established on the biosensor surface. 8000 relative response units of a goat anti-human Fcγ fragment-specific polyclonal antibody (Jackson Laboratories) were immobilized according to the manufacturer's instructions using EDC/NHS chemistry. The sensor was deactivated using 1M ethanolamine.
20 nM of the respective antibody sample were captured for 1 min at a flow rate of 10 µl/min. As a reference, 20 nM polyclonal human normal IgG (Roche, Ident. 11717570) were captured on the reference flow cell 1 and subtractive signals were monitored.
In one embodiment, the 23.3 kDa analyte DR5 was injected at 30 µl/min for 3 min association time in concentration series at 0 nM, 3.3 nM, 11 nM, 2 x 33 nM, 100 nM and 300 nM. The complex dissociation was monitored for 5 min.
The system was regenerated at 30 µl/min by a 1 min injection of 10 mM glycine buffer pH 1.5 followed by a two consecutive 1 min injections of 10 mM glycine buffer pH 1.7. Kinetic parameters were evaluated using the Biacore Evaluation Software according to the manufacturer's instructions.
The association rate constant k_a(1/Ms), the dissociation rate constant k_d (1/s) and the dissociation constant K_D were calculated according to a Langmuir model with R_{MAX} global.

Table 25: Kinetic affinities to monomeric human DR5 of humanized variants of DR5TAA-0011 (DR5TAA-0067 to DR5TAA-0075) as compared to chimeric form of DR5TAA-0011 (DR5TAA-0052)

Construct	SEQ ID No VH/VL	ka (1/Ms)	kd (1/s)	KD (nM)
DR5TAA-0067	23/24	2,6E05	5.2E-03	20
DR5TAA-0074	23/31	2.7E05	5.6E-03	21
DR5TAA-0071	26/24	3.0E05	4.6E-03	15
DR5TAA-0075	23/29	3.2E05	5.6E-03	17
DR5TAA-0052	90/92	3.5E05	2.4E-03	7

Kinetic binding properties of humanized variants were maintained within a factor of approx. 3 for KD and kd and within a factor of approx. 2 for ka as compared to the chimeric variant.

Example 34: Characterization of thermal and chemical stability of humanized DR5-binder derived by immunization

Humanized DR5-binder were characterized for their thermal and chemical stability. Samples were prepared at a concentration of 1 mg/mL in 20 mM Histidine/Histidine chloride, 140 mM NaCl, pH 6.0, transferred into an optical 384-well plate by centrifugation through a 0.4 µm filter plate and covered with paraffin oil. The hydrodynamic radius is measured repeatedly by dynamic light scattering while the samples are heated with a rate of 0.05 °C/min from 25 °C to 80 °C. The aggregation onset temperature is defined as the temperature at which the hydrodynamic radius starts to increase.

Table 26: Aggregation Onset temperatures of humanized variants of DR5TAA-0011 (DR5TAA-0066-DR5TAA-0075) as a measure for thermal stability of the antibodies.

Construct	SEQ ID No VH/VL	Tagg [°C]
DR5TAA-0071	26/24	71
DR5TAA-0067	23/24	70
DR5TAA-0074	23/31	68
DR5TAA-0073	26/30	72
DR5TAA-0068	26/31	69
DR5TAA-0072	26/32	71
DR5TAA-0066	23/30	71
DR5TAA-0075	23/29	68

Humanized variants of DR5TAA-0011 reveal a high thermal stability with aggregation temperatures of 68 °C and higher.

Chemical Stability

Generation of stressed DR5 antibody samples: To test the chemical stability of DR5 antibodies, stressed samples were generated and functionally characterized with regard to DR5 binding. High-pH stress induces – among others – deamidation of reactive Asn hotspots, whereas at pH 6.0 e.g. succinimide formation from reactive Asn and Asp residues may be induced. For high-pH stress, samples were transferred in 20 mM Na-phosphate, pH 8.0 and incubated for 5 days at 40°C. For low-pH stress, samples were transferred into in 20 mM His/HisCl, 140 mM NaCl, pH 6.0 and incubated for 3 weeks at 40°C. A control sample was kept at -80°C.

Analysis of stressed DR5 antibody samples: A BIAcore 3000 instrument (GE Healthcare) was used with a CM5 sensor mounted into the system. The sensor was preconditioned by a 1 min injection at 100 µl/min of 0.1 % SDS, 50 mM NaOH, 10 mM HCl and 100 mM H3PO4. System buffer was 10 mM HEPES pH 7.4, 150 mM NaCl, 0.05% TWEEN 20. The sample buffer was the system buffer supplemented with 1 mg/mL carboxymethyldextran (Sigma). An anti-human antibody capture system was established on the biosensor surface. 8000 relative response units of a goat anti-human Fcγ fragment-specific polyclonal antibody (Jackson Laboratories) were immobilized according to the manufacturer's instructions using EDC/NHS chemistry. The sensor was deactivated using 1M ethanolamine.

20 nM of the respective antibody sample were captured for 1 min at a flow rate of 10 µl/min. As a reference, 20 nM polyclonal human normal IgG (Roche, Ident. 11717570) were captured on the reference flow cell 1 and subtractive signals were monitored.

In another embodiment, the analyte was injected at 100 µl/min for 2 min association time at 0 nM and 500 nM. The complex dissociation was monitored for 5 min.

Table 27: Relative active concentrations of humanized variants of DR5TAA-0011 before and after stress test

Construct	SEQ ID No VH/VL	Initial (defined)	pH 6.0 2 weeks 40°C Relative active concentration as compared to the initial state	pH 7.4 2 we 40°C Relative active concentration as compared to the initial state
DR5TAA-0071	26/24	100%	103%	98%
DR5TAA-	23/24	100%	107%	107%

0067				
DR5TAA-0074	23/31	100%	103%	100%
DR5TAA-0073	26/30	100%	104%	101%
DR5TAA-0068	26/31	100%	103%	101%
DR5TAA-0072	26/32	100%	105%	101%
DR5TAA-0066	23/30	100%	102%	100%
DR5TAA-0075	23/29	100%	102%	99%

The system was regenerated at 30 µl/min by a 1 min injection of 10 mM glycine buffer pH 1.5 followed by a two consecutive 1 min injections of 10 mM glycine buffer pH 1.7. Kinetic parameters were evaluated using the Biacore Evaluation Software according to the manufacturer's instructions.

The antibody/antigen complex half-life was calculated in minutes according to the formula ln(2)/(60**kd)*. The Molar Ratio was calculated: MW (antibody) / MW (antigen) *BL (antigen)/ CL (antibody).

Data report points were recorded shortly before the end of the antibody injection (antibody capture level, CL) as well as shortly before the analyte (Binding Late, BL) injection. Capture Level (CL) and Binding Late (BL) response signal heights were used to characterize the antibody binding performance. The relative binding quotient was calculated BL/CL. A quotient was formed from the relative binding quotient of an antibody sample versus a non-stress impacted antibody sample (relative active binding).

Results are shown in Table 27. Humanized variants of DR5TAA-0011 were subjected to stress test. None of the humanized variants showed impaired binding to human DR5 as compared to the non-stressed initial material.

Example 35: Materials and Methods

Unless otherwise mentioned the following materials and methods have been used in the experiments outlined above.

Recombinant DNA technologies

All antibody and antigen expression vectors were generated using standard recombinant DNA technology as described in Sambrook, J. et al., Molecular cloning: A laboratory manual;

Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. Molecular biological reagents were used according the manufacturer's recommendations. Genes or gene fragments were either amplified by polymerase chain reaction (PCR) or generated from synthetic oligonucleotides at Geneart AG (Regensburg, Germany) by automated gene synthesis. PCR-amplified or subcloned DNA fragments were confirmed by DNA sequencing (Synergene GmbH, Switzerland). Plasmid DNA was transformed into and amplified in suitable *E. coli* host strains for preparation of transfection-grade plasmid DNA using standard Maxiprep kits (Qiagen). For production of the bispecific molecules HEK293 EBNA cells were transfected with plasmids encoding the respective genes using a standard polyethlenimine (PEI) based method. The used plasmid ratio of the three expression vectors was 1:1:1. Transfected cells were cultivated for 7 days before supernatants were harvested for purification.

Transfection HEK293 EBNA cells

All (bispecific) antibodies and antigens (if not obtained from a commercial source) used herein were transiently produced in HEK 293 EBNA cells using a PEI mediated transfection procedure for the required vectors as described below.

HEK293-EBNA cells are cultivated in suspension serum free in CD CHO culture medium. For the production in 500 ml shake flask 400 million HEK293- EBNA cells are seeded 24 hours before transfection. For transfection cells are centrifuged for 5 min by 210 x g, supernatant is replaced by pre-warmed 20 ml CD CHO medium. Expression vectors are mixed in 20 ml CD CHO medium to a final amount of 200 µg DNA. After addition of 540 µl PEI solution is vortexed for 15 s and subsequently incubated for 10 min at room temperature. Afterwards cells are mixed with the DNA/PEI solution, transferred to a 500 ml shake flask and incubated for 3 hours by 37 °C in an incubator with a 5 % CO2 atmosphere. After incubation time 160 ml F17 medium is added and cell are cultivated for 24 hours. One day after transfection 1 mM valporic acid and 7 % Feed 1 is added. After 7 days cultivation supernatant is collected for purification by centrifugation for 15 min at 210 x g, the solution is sterile filtered (0.22 µm filter) and sodium azide in a final concentration of 0.01 % w/v is added, and kept at 4°C.After production the supernatants were harvested and the antibody containing supernatants were filtered through 0.22 µm sterile filters and stored at 4 °C until purification.

Purification: standard + ProtA beads

The proteins were produced by transient expression in HEK293 EBNA cells. All bispecific molecules described here were purified in two steps using standard procedures, such as proteinA affinity chromatography (Äkta Explorer) and size exclusion chromatography (Superdex 200).

The supernatant was adjusted to pH 8.0 (using 2 M TRIS pH 8.0) and applied to Mabselect Sure resin (GE Healthcare) packed in a Tricorn™ 5/50 column (GE Healthcare, column volume (cv) = 1 ml) equilibrated with buffer A (50 mM sodiumphosphate, pH 7.0, 250 mM NaCl).

Unbound protein was removed by washing with at least 5 column volumes (CV) of buffer A. The protein of interest was eluted in a linear pH-gradient from 0-100% buffer B (50 mM sodiumphosphate, pH 7.0, 1 M NaCl) over 12 CV. Finally, an additional step was included with 10 CV of buffer B followed by an equilibration step of 5 CV buffer A. Fractions containing the protein of interest were pooled and the pH was gradually adjusted to pH 6.0 (using 2 M TRIS pH 8.0). Samples were concentrated to 0.5-2 ml using ultra-concentrators (Vivaspin 15R 30.000 MWCO HY, Sartorius) and subsequently applied to a HiLoad™ 16/60 Superdex™ 200 preparative grade column (GE Healthcare) equilibrated with 20 mM Histidine, pH 6.0, 140 mM NaCl, 0.01% Tween-20. The aggregate content of eluted fractions was analyzed using a TSKgel G3000 SW XL analytical size-exclusion column (Tosoh) equilibrated in 25 mM K2HPO4, 125 mM NaCl, 200mM L-Arginine Monohydrocloride, 0.02 % (w/v) NaN3, pH 6.7 at 25°C. Fractions containing less than 2 % oligomers were pooled and concentrated to a final concentration of 1 - 1.5 mg/ml using ultra concentrators (Vivaspin 15R 30.000 MWCO HY, Sartorius). Purified proteins were frozen in liquid N_2 and stored at -80 °C.

For a fast and high throughput purification, supernatants were neutralized using 1/40th column of 2M Tris-HCl pH8 and incubated with ProteinA Sepharose Fast Flow beads (GE Healthcare Cat No. 17-5138-01)) for 19h at 4°C end over end. The supernatant/bead mixture was then passed over an empty, equilibrated PD-10 column (GE Healthcare Cat No. 17-0435-01) by gravity flow. The retained beads were washed twice with binding buffer (10 mM Tris, 50 mM glycine, 100 mM NaCl, pH 8.0) and the antibody eluted with a low pH step (10 mM Tris, 50 mM glycine, 100 mM NaCl, pH2.5). Finally the eluted protein was neutralized by addition of 1/40th volume of 2M Tris-HCl pH8.0. The protein concentration of purified antibodies was calculated from the measured absorbance at 280 nm and the molar extinction coefficient calculated from the amino acid sequence. The aggregate content of the antibody sample was analysed using a Zorbax GF-250 analytical size exclusion column (Agilent Cat No PSMO 845006) equilibrated in running buffer (200 mM sodium phosphate, 0.02% sodium azide pH 7.0) at 25°C.

FACS binding analysis

All used target cell lines were analyzed for relative expression levels of tumor-related antigens and DR5 death receptors before apoptosis assays were performed.

Number and viability of cells was determined. For this, adherently growing cells were detached with cell dissociation buffer (Gibco – Invitrogen # 13151-014). Cells were harvested by centrifugation (4 min, 400 x g), washed with FACS buffer (PBS / 0.1 % BSA) and the cell number was adjusted to 1.111 X 10^6 cells / ml in FACS buffer. 180 µl of this cell suspension was used per well of a 96 well round bottom plate, resulting in 2 x 10^5 cell per well. The cells were incubated for 30 min at 4 °C with the first antibody in appropriate dilution. Then the cells were harvested by centrifugation (4 min, 400 x g), supernatant was completely removed and cells were washed once with 150 µl of FACS buffer. The cells were resuspended in 12 µl diluted

secondary antibody (in case unlabelled first antibody was used) or FACS buffer for 30 min at 4 °C in the dark. After two washing steps with FACS buffer cells were resuspended in 200 µl of FACS buffer and analyzed in a HTS FACSCanto II (BD, Software FACS Diva). Alternatively the cells could be fixed with of 200 µl of 2 % PFA (paraformaldehyde) in FACS buffer for 20 min at 4 °C and analyzed later. All assays were performed in triplicates.

Table 28: Antibodies and concentrations for FACS binding analysis

Antibody	Source	Description	Conc. [mg / ml]	Conc. in test [µg / ml]
1. First antibodies				
anti hu DR5 (TRAIL R2)	R&D #MAB631	mu IgG1, clone 71903	0.5	5 - 10
Drozitumab	in house	hu IgG1	3.8	10
4G8	in house	hu IgG1	20.5	15
mouse anti-human FAP	Calbiochem #OP188	mu IgG1	1	10
Drozitumab-scFab-FAP	in house		1.34	25
Drozitumab-X-FAP_A			1.44	25
Drozitumab-X-FAP_B			0.83	25
5E11_28H1_N-term VHVL			2.55	as indicated
5E11_28H1_N-term CH1CL			1.12	as indicated
5E11_28H1_C-term CH1CL			4.0	as indicated
2. Secondary antibodies:				
PE-conjugated AffiniPure F(ab')2 Fragment goat anti-human IgG Fcg Fragment Specific	Jackson ImmunoResearch Lab # 109-116-170			1:20 dilution
FITC-conjugated F(ab')2 goat anti-mouse IgG Specific	Serotec # STAR105F			1:20 dilution
FITC-conjugated AffiniPure F(ab')2 Fragment goat anti-human IgG Fcg Fragment Specific	Jackson Immuno Research Lab # 109-096-098			1:20 dilution

Biacore analysis (Surface Plasmon Resonance, SPR)

Binding of the various anti-DR5 binders as IgG or in a bispecific format was assessed by surface plasmon resonance (SPR). All SPR experiments were performed on a Biacore T100 at 25°C with HBS-EP as running buffer (0.01 M HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20, Biacore, Freiburg/Germany).

For the determination of the species cross-reactivity of the DR5 binders, biotinlyated DR5 from mouse, human and cynomolgus were directly coupled to different flow cells of a Streptavidin (SA) sensor chip with an immobilization level of approximately 300 RU each. The various DR5 binders as IgGs were injected at a concentration of 500, 100 and 25 nM for 60 s with a flow rate of 30ul/min, followed by a dissociation phase of 90 s. Bulk refractive index differences were corrected for by subtracting the response obtained on reference flow cell, where no protein was immobilized.

In a second experiment, the specificity of the DR5 binders was determined by immobilizing huDR4Fc, huDcR1Fc, huDcR2Fc and rhuOPGFc to a CM5 sensor chip by amine coupling. Immobilisation levels were between 100 and 400 RU. Each binder was passed over the different flow cells at a concentration of 500, 100 and 25 nM for 60 s with a flow rate of 30 µl/min and the dissociation phase monitored for 90 s. Bulk refractive index differences were corrected for by subtracting the response obtained on the reference flow cell, where no protein was immobilized.

Further, the avidity of the IgGs as well as the 2+2 formats was measured on a CM5 chip with immobilized human and cynomolgus DR5 ECD (immobilization levels were around 100 RU). Each construct or IgG was passed over the different flow cells at a concentration between 500-0.97 nM in 1:2 dilution steps for 90s at 30 µl/min. The dissociation was analysed for 120 s. Bulk refractive index differences were corrected for by subtracting the response obtained on the reference flow cell, where no protein was immobilized. Kinetic constants were calculated using the Biacore T100 Evaluation Software (vAA, Biacore AB, Uppsala/Sweden), to fit rate equations for 1:1 Langmuir binding by numerical integration either as kinetic analysis or steady state analysis.

Affinity was assessed using a capture format where either an anti-human Fab or anti-human Fc antibody (Biacore, Freiburg/Germany) was directly coupled on a CM5 chip at pH 5.0 using the standard amine coupling kit (Biacore, Freiburg/Germany). The immobilization level was about 8,000-10000 RU. DR5 binders in an IgG or 2+2 format were captured at a concentration of 50 or 30 nM respectively for 60 s at a flow rate of 30 µl/min. Injection of human or cynomolgus DR5 in a concentration range from 500 - 0.975 nM (cynomolgus DR5) or 1000 - 0.975 nM (huDR5) in 1:2 dilution steps for 120 s at 30 µl/min was carried out for the 2+2 format. Affinity of the IgGs was only measured for huDR5 at a concentration from 2000 - 20 nM in 1:3 dilution steps. Dissociation was evaluated over a period of 120 s. Bulk refractive index differences were corrected for by subtracting the response obtained on reference flow cell, where no protein was immobilized. Kinetic constants were calculated using the Biacore T100

Evaluation Software (vAA, Biacore AB, Uppsala/Sweden), to fit rate equations for 1:1 Langmuir binding by numerical integration either as kinetic analysis or steady state analysis.

Epitope binning was measured in two different formats, either with a classical sandwich assay or with a tandem approach. In the classical sandwich assay, each DR5 binders is directly immobilized by amine coupling to one flow cell on the CM5 chip surface with a target immobilization level of around 500 RU. Subsequently, huDR5 is passed over each flow cell at a concentraion of 500 nM for 60 s (flow rate 30 µl/min) followed by an injection of another DR5 binder at a concentration of 30 nM for 60 s (flow rate 30 µl/min). The dissociation is monitored over a period of 60 s with the same flow rate. Injection of the same DR5 binder than the immobilized one is used as a control as this should not lead to a response increase if all DR5 is bound by the immobilized binder.

In the tandem approach, huDR5ECD was immobilized on a CM5 chip with a final response of 250 RU. The first DR5 binder was then passed over the flow cell at a concentration of 20 nM for 90 s, followed by the injection of a second DR5 binder for 90 s. The dissociation was monitored over a period of 90 s. The flow rate was 30 µl/min for all steps. If the two binders recognize a different epitope, one could observe an increase in the response units. Injection of the same DR5 binder was used as a control to confirm that all DR5 molecules were saturated by the first injection and no additional binding to the same epitope can occur.

To further determine if any of the binders are ligand blocking, rhuTRAIL (Peprotech Cat No. 310-04) was immobilized on a CM5 chip by amine coupling with an immobilization level of 2000 RU. huDR5 Fc or huDR5 ECD were complexed with each DR5 binder to be tested (100 nM DR5 with 500 nM IgG) and the complex passed over the flow cell for 90 s with a flow rate of 50 µl/min. The dissociation was assessed over a period of 120 s. In addition, a classical sandwich assay was used, where huDR5Fc or huDR5 ECD was injected first at 100 nM followed by an injection of each DR5 binder at 500 nM. Contact times were 60 s for DR5 and 90 s for the IgGs with a flow rate of 30 µl/min. The dissociation step was carried out for 90s. Binders which could bind to TRAIL, in addition to DR5, were considered to be non-ligand blocking whereas binders which did not show any additional bind as ligand blocking.

Simultaneous binding of the various DR5 binders in a 2+2 format was confirmed on a SA chip containing immobilized huDR5Fc biotin (immobilisation level around 1000 RU). In a first step, the 2+2 construct was injected for 90 s followed by an injection of either human or murine FAP at a concentration of 500 or 100 nM for 90 s. The dissociation was monitored for 60 s. The flow rate for all steps was 30 µl/min. Simultaneous binding was considered to be true if an additional increase in response units was observed upon injection of human or murine FAP.

Epitope binning was measured in two different formats either a classical sandwich assay or with a tandem approach. In the classical sandwich assay each DR5 binders gets directly immobilized by amine coupling to one flow cell on the CM5 chip surface with a target immobilization level of around 500 RU. Then, huDR5 is passed over each flow cell at a conc. of

500 nM for 60 s (flow rate 30 ul/min) followed by an injection of another DR5 binder at a concentration of 30 nM for 60 s (flow rate 30 ul/min). The dissociation is monitored over a period of 60 s with the same flow rate. Injection of the same DR5 binder than the immobilized one is used as a control as this should not lead to a response increase if all DR5 is bound by the immobilized binder.

In the tandem approach huDR5ECD was immobilized on a CM5 chip with a final response of 250 RU. The first DR5 binder is then passed over the flow cell at a concentration of 20 nM for 90 s, followed by the injection of a second DR5 binder for 90 s. The dissociation was monitored over a period of 90 s. The flow rate was 30 ul/min for all steps. If the two binders recognize a different epitope, one could observe an increase in the response units. Injection of the same DR5 binder was used as a control to confirm that all DR5 molecules were saturated by the first injection and no additional binding to the same epitope can occur.

To further determine if any of the binders are ligand blocking rhuTRAIL (Peprotech Cat No. 310-04) was immobilized on a CM5 chip by amine coupling with an immobilisation level of 2000 RU. huDR5 Fc or huDR5 ECD were complexed with each DR5 binder to be tested (100 nM DR5 with 500 nM IgG) and the compley was passed over the flow cell for 90 s with a flow rate of 50 ul/min. The dissociation was assessed over a period of 120 s. In addition, a classical sandwich assay was used, where huDR5Fc or huDR5 ECD was injected first at 100 nM followed by an injection of each DR5 binder at 500 nM. Contact times were 60 s for DR5 and 90 s for the IgGs with a flow rate of 30 ul/min. The dissociation step was carried out for 90s. Binders which could bind in addition of DR5 to TRAIL were considered to be non-ligand blocking whereas binders which did not show any additional bind as ligand blocking.

Simultaneous binding of the various DR5 binders in a 2+2 format was confirmed on a SA chip containing immobilized huDR5Fc biotin (immobilisation level around 1000 RU). In a first step, the 2+2 construct was injected for 90 s followed by an injection of either human or murine FAP at a concentration of 500 or 100 nM for 90 s. The dissociation was monitored for 60 s. The flow rate for all steps was 30 µl/min. Simultaneous binding was considered to be true if an additional increase in response units was observed upon injection of human or murine FAP.

DNA fragmentation ELISA

For determination of induced apoptosis the Cell Death Detection ELISA ^{PLUS} kit from Roche was used. In short, 10^4 FAP expressing GM05389 cells per well of a 96-well plate (after detaching, and determination of cell number and viability) were seeded in 200 µl appropriate medium and were incubated over night at 37 °C in a 5 % CO_2 atmosphere. The next day the medium was replaced by 100 µl of fresh medium containing the apoptosis inducing antibodies, control antibodies and other controls in appropriate concentrations:

The bispecific antibodies and IgGs were used in a final concentration of 0.7 and 7 nM or as indicated; cross-linking antibodies were used at the same molarity as the primary antibodies. After addition of the antibodies10^4 apoptosis sensitive tumour cells were added per well.

The cells were incubated for 24 hrs at 37 °C, 5 % CO_2 to allow induction of apoptosis. The cells were harvested by centrifugation (10 min, 200 x g) and incubated for 1 h at room temperature in 200 µl of lysis buffer (supplied by the kit). Intact DNA and the lysed cells were sedimented by centrifugation (10 min, 200 x g) and 20 µl of the supernatant containing the fragmented DNA was analyzed according to the manufacturer's recommendations for induction of apoptosis.

Inhibition of proliferation (CellTiterGlo)

For Cell Viability assays 4,000 tumor cells /well (in 75µl volume) were seeded in black, clear bottom 96 well plates (BD Falcon cat#BD353220) and incubated overnight at 37^0C in a humidified, 5% CO_2 atmosphere. Then 25µl 4x DR5 binder plus/minus rabbit-Fc (equal nano molar of DR5 binders and Fc) with 6 concentrations of 2.5 x serial dilutions were added. After incubation at 37^0C with 5% CO_2 for 48 hours 100 µl CellTiter-Glo reagent was added to each well and mixed well. After incubation for another 10 minutes at room temperature results cell viability was determined with a Spectra Max M5 plate reader under luminescence settings.

Induction of Caspase 8 (Caspase Glo)

For Caspase 8 activation assays 10,000 cancer cells/well in 75 µl were seeded in opaque white 96 well plates (BD Falcon cat#BD353296) and incubated at 37^0C in a humidified incubator with 5% CO_2 overnight. Then 25ul 4x DR5 binders plus/minus anti-Fc were added (equimolar ratios of DR5 binders and anti Fc) with 6 concentrations of 2.5x serial dilutions. After 3 hours incubation at 37^0C with 5% CO_2 100 µl caspase 8 substrate in lysis buffer was added to each well. After additional incubation for 30 minutes at 37^0C the results were red in a Spectra Max M5 plate reader under luminescence settings.

FRET assays

The binding of bispecfic molecules on cells was determined using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay (called TagLite). Hek EBNA cells were grown to 60-80% confluency and transfected with plasmid DNA encoding for DR5 ECD fused to a SNAP Tag and the MalE TM. Briefly, 2 µg DNA was mixed with 4 ml OptiMEM medium and 30 µl Lipofectamine 2000 (Invitrogen Cat No. 11668-019). The mixture was incubated for 20 min at RT. In the meantime, the adherent Hek EBNA cells grown in a T75 flask were washed with 5 ml D-PBS prior to adding the transfection mixture and culture medium (6 ml) (DMEM, 10% FCS, glutamax, Non-essential amino acids). The cells were then incubated overnight in a humidified incubator (5% CO2) at 37°C. Cells were washed with 5 ml D-PBS, followed by the addition of a mixture of 5 ml TagLite buffer (Cisbio Cat No.) containing 100 nM

SNAP-Lumi4-Tb (Cisbio Cat No.). This resulted in attachment of the fluorescent dye to the SNAP Tag fused to the DR5. After an incubation time of 1 h at 37°C, the cells were washed with TagLite buffer to remove unbound dye. Subsequently, the labelling efficiency was checked by measuring the fluorescent signal at 620 nm (excitation 343 nm) of 10000 cells in a 384 well format (Reader, Victor, Perkin Elmer). Cells were then frozen in culture medium substituted with 10% DMSO and stored at -80°C.

To carry out a binding assay, pre-labeled cells were thawed, washed and 1000 cells per well mixed with 5 µl construct at a final concentration ranging from 50 - 0.097 nM (1:2 dilution steps) and 5 µl anti-huFc-d2 labeled (final concentration 150 nM). The fluorescent signal was measured at 620 nm for the fluorescent donor (Terbium) and at 665nm for the fluorescent acceptor dye after 0h, 1h and 3h incubation at RT. The ratio of 665/620*1000 was calculated, and the reference (cells with 150 nM anti-huFc-d2) was subtracted. For KD determination the results were analysed in Graph Pad Prism with one site fit-specific binding.

Determination of the thermal stability by Dynamic Light Scattering (DLS)

Thermal stability of the protein is monitored by Dynamic Light Scattering (DLS). 30 µg of filtered protein sample with a protein concentration of 1 mg/ml is applied in duplicate to a Dynapro plate reader (Wyatt Technology Corporation; USA). The temperature is ramped from 25 to75 °C at 0.05 °C/min, with the radius and total scattering intensity being collected.

Table 29: Names and Aliases of DR5 Clones and Bispecific Constructs

DR5-FAP bispecific construct	DR5 Clone (SEQ ID VH/VL)	FAP Clone (SEQ ID VH/VL)	Format	Complete antibody sequence	Scetch of Format
DR5TAA-0061	DR5TAA-0011 (SEQ ID NO.:88/89)	4B9 (SEQ ID NO.:39/40)	1 + 1	SEQ ID NOs 280,281,282,283	See Fig. 25 D
DR5TAA-0030	5E11 (SEQ ID NO.:7/8)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 131,132,124	See Fig. 28 A
DR5TAA-0032	DR5TAA-0005 (SEQ ID NO.:41/46)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 284,285,286	See Fig. 28 A
DR5TAA-0033	DR5TAA-0011 (SEQ ID NO.:88/89)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 287,288,289	See Fig. 28 A
DR5TAA-0034	DR5TAA-0013 (SEQ ID NO.:68/71)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 290,291,292	See Fig. 28 A
DR5TAA-0035	DR5TAA-0016 (SEQ ID NO.:82/85)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 293,294,295	See Fig. 28 A
DR5TAA-0036	DR5TAA-0019 (SEQ ID NO.:74/78)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 296,297,298	See Fig. 28 A

DR5TAA-0037	22E9 (SEQ ID NO.:100/101)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 124,125,126	See Fig. 28 A
DR5TAA-0038	21H3 (SEQ ID NO.:102/103)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 127,128,124	See Fig. 28 A
DR5TAA-0039	20F2 (SEQ ID NO.:106/107)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 129,130,124	See Fig. 28 A
DR5TAA-0055	5E11 (SEQ ID NO.:7/8)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 133, 132, 124	See Fig. 28 A
DR5TAA-0057	5E11 (SEQ ID NO.:7/8)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 134,132,124	See Fig. 28 A
DR5TAA-0058	5E11 (SEQ ID NO.:7/8)	4B9 (SEQ ID NO.:39/40)	2 + 2	SEQ ID NOs 262,263,132	See Fig. 28 A
DR5TAA-0077	5E11 (SEQ ID NO.:7/8)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 135,136,137	See Fig. 28 C
DR5TAA-0078	5E11 (SEQ ID NO.:7/8)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 138, 139,137	See Fig. 28 D
DR5TAA-0081	5E11 (SEQ ID NO.:7/8)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 278,132,279	See Fig. 28 F
DR5TAA-0117	DR5TAA-0067 (SEQ ID NO.:23/24)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 299,300,301	See Fig. 28 A
DR5TAA-0118	DR5TAA-0071 (SEQ ID NO.:26/24)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 302,303,281,282, 283	See Fig. 28 A
DR5TAA-0119	DR5TAA-0075 (SEQ ID NO.:23/29)	28H1 (SEQ ID NO.:15/16)	2 + 2	SEQ ID NOs 305,306,307	See Fig. 28 A

DR5 Antibody Clone Name	**DR5 Antibody Clone Alias**
DR5TAA-0005	„0005“ or „039“
DR5TAA-0006	„0006“ or „058“
DR5TAA-0010	„0010“ or „481“
DR5TAA-0013	“0013” or “298”
DR5TAA-0019	“0019” or “461”
DR5TAA-0016	“0016” or “422”
DR5TAA-0011	“0011” or “174”

Example 36: In vivo antitumor efficacy of DR5-FAP bispecific antibody comprising newly isolated DR5 binder 5E11 in combination with 28H1 FAP CrossFab

The in vivo antitumor efficacy of the bispecific antibody DR5-FAP (VH SEQ ID NO.:7, VL SEQ ID NO.: 8) was demonstrated in cell and fragment based patient derived (PDX) models of various tumor origin (e.g. CRC and pancreatic cancer) transplanted on nude mice. As example data are shown for the CRC xenograft model DLD-1 (cell line based, co-injection model) and Co5896 (fragment based).

Test agents

The bispecific antibody DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) was provided as stock solution from Roche, Penzberg, Germany. Antibody buffer included histidine. Antibody solution was diluted appropriately in buffer from stock prior injections.

Cell lines and culture conditions

DLD-1 human CRC cells were originally obtained from ATCC. The tumor cell line was routinely cultured in DMEM high glucose medium with 1.0mM Sodium pyruvate supplemented with 10% fetal bovine serum, 2.0mM L-glutamine, 10mM HEPES at 37 °C in a water-saturated atmosphere at 5% CO_2. Culture passage was performed with trypsin / EDTA 1x splitting every third day. Additionally murine fibroblasts NIH3T3 were purchased from ATCC and cultured in DMEM high glucose with 1.0mM Sodium pyruvate, FCS 10% and L-Glutamine 2.0mM.

Patient-derived xenograft model (PDX)

The CRC tumor xenograft Co5896 was originally obtained from patients and passaged approximately three to five times until establishment of stable growth patterns. For the subsequent in vivo studies Co5896 tumor fragments were obtained from xenografts in serial passage in nude mice. After removal from donor mice, tumors were cut into fragments (4-5 mm diameter) and placed in PBS until subcutaneous implantation. Mice under isofluorane anesthesia received unilateral, subcutaneous tumor implants in the flank.

Animals

Nude mice were purchased from breeder (e.g. Charles River, Sulzfeld, Germany) and maintained under specific-pathogen-free condition with daily cycles of 12 h light /12 h darkness according to committed guidelines (GV-Solas; Felasa; TierschG). Experimental study protocol was reviewed and approved by local government. After arrival animals were maintained in the quarantine part of the animal facility for one week to get accustomed to new environment and for observation. Continuous health monitoring was carried out on regular basis. Diet food (Provimi Kliba 3337) and water (acidified pH 2.5-3) were provided ad libitum.

Monitoring

Animals were controlled daily for clinical symptoms and detection of adverse effects. For monitoring throughout the experiment body weight of animals was documented.

Treatment of animals

Animal treatment started after animal randomisation after cell or fragment transplantation when median tumor size was about 100–200mm^3. Antibody was administered as single agent at 10 or 30mg/kg i.v. once or twice weekly for several weeks depending on the model. The corresponding vehicle was administered on the same days.

Antibody efficacy

DLD-1 CRC co-injection cell line based xenograft model

DLD-1 CRC xenograft bearing mice were treated with bispecific antibody DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) from study day 9 to 20 at dosages of 10 and 1.0mg/kg for 4 times. As a result, treatment with bispecific antibody DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) showed dose-related significant anti-tumor efficacy with strong anti-tumor efficacy against s.c. DLD-1 xenografts. The Tumor Growth Inhibition (TGI) was calculated at 89% (10mg/kg) and 79% (1.0mg/kg), respectively. In contrast, after treatment with DR5 Fc mutant antibody drozitumab PG, LALA (10mg/kg, once weekly) no anti-tumor efficacy was noticed (see Figure 48). Similar results were obtained with high FAP content (Co-injection study with DLD-1 / NIH3T3 fibroblasts; Ratio 80/20, Figure 48) and low FAP content (Co-injection study with DLD-1 / MRC5 fibroblasts; Ratio 30/70, data not shown).

Co5896 CRC fragment based patient derived xenograft model (PDX)

Co5896 CRC xenograft bearing mice were treated with bispecific antibody DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) from study day 18 to 34 at dose of 30mg/kg for 6 times. As a result, treatment with bispecific antibody DR5-FAP (DR5 binder: VH SEQ ID NO.:7, VL SEQ ID NO.: 8, FAP binder: VH SEQ ID NO.:15, VL SEQ ID NO.: 16) showed significant anti-tumor efficacy with strong anti-tumor efficacy against s.c. Co5896 patient-derived xenografts. The Tumor Growth Inhibition (TGI) was calculated at 76% (see Figure 49). Similar results were obtained in other CRC cell models (data not shown).

Example 36: FAP prevalence in human tumors

The prevalence of FAP in human tumors was evaluated by IHC to get an understanding on possible clinical use of bispecific DR5-FAP antibody.

Rat anti-human Seprase antibody (IgG2a, clone D8) from Vitatex (MABS1001) was used to immunostain 2,5 µm FFPET sections from various tumour indications on the Ventana Benchmark XT. Sections were subjected to standard CC1 treatment followed by antibody incubation for 60' at 37°C at a concentration of 5 µg/mL in Dako antibody diluent (S3022) and positive staining was detected using the Ultraview DAB detection system (Ventana #760-4456). Matched isotype antibody from Abcam (ab18450) was used as the negative control.

FAP+ stromal infiltrate was present in human tumors of different indications including head and neck squamous cell carcinoma (HNSCC), breast cancer, colorectal cancer (CRC), pancreatic cancer (PAC), gastric cancer, non-small-cell lung carcinoma (NSCLC) and Mesothelioma marking potentially interesting clinical indications for a bispecific DR5-FAP antibody (Table 30).

Table 30: FAP prevalence in human tumors

Tumor Type	% cases with **moderate to high** grade of **FAP$^+$ infiltrate**	**n** of samples investigated
HNSCC	90	10
Breast Cancer	77	105
triple negative BC	80	7
CRC	77	90
PAC	74	19
Gastric Cancer	68	28
NSCLC	66	90
Mesothelioma	60	10

Sequences

1. Amino acid sequences of phage display derived DR5 binders

Description	**Amino acid sequence**	**SEQ ID NO.**
DR5 (22E9)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKGVRISFDYWGQGT LVTVSS	100
DR5 (22E9)_CDRH1	SYAMS	1

Description	Amino acid sequence	SEQ ID NO.
DR5 (22E9)_CDRH2	AISGSGGSTYYADSVKG	2
DR5 (22E9)_CDRH3	GVRISFDY	96
DR5 (22E9)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGSNQPVTFGQGTKVEIK	101
DR5 (22E9)_CDRL1	RASQSVSSSYLA	4
DR5 (22E9)_CDRL2	GASSRAT	5
DR5 (22E9)_CDRL3	QQGSNQPVT	99
DR5 (21H3)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDMAVYYCAKGARVSFDYWGQG TLVTVSS	102
DR5 (21H3)_CDRH1	SYAMS	1
DR5 (21H3)_CDRH2	AISGSGGSTYYADSVKG	2
DR5 (21H3)_CDRH3	GARVSFDY	104
DR5 (21H3)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGSQPPITFGQGTKVEIK	103
DR5 (21H3)_CDRL1	RASQSVSSSYLA	4
DR5 (21H3)_CDRL2	GASSRAT	5
DR5 (21H3)_CDRL3	QQGSQPPIT	105
DR5 (20F2)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKGVRKGFDYWGQG TLVTVSS	106
DR5 (20F2)_CDRH1	SYAMS	1
DR5 (20F2)_CDRH2	AISGSGGSTYYADSVKG	2
DR5 (20F2)_CDRH3	GVRKGFDY	108
DR5 (20F2)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGESPPPTFGQGTKVEIK	107
DR5 (20F2)_CDRL1	RASQSVSSSYLA	4

Description	Amino acid sequence	SEQ ID NO.
DR5 (20F2)_CDRL2	GASSRAT	5
DR5 (20F2)_CDRL3	QQGESPPPT	109
DR5 (5E11)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKGVRVSFDYWGQGT LVTVSS	7
DR5 (5E11)_CDRH1	SYAMS	1
DR5 (5E11)_CDRH2	AISGSGGSTYYADSVKG	2
DR5 (5E11)_CDRH3	GVRVSFDY	3
DR5 (5E11)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGTTHPITFGQGTKVEIK	8
DR5 (5E11)_CDRL1	RASQSVSSSYLA	4
DR5 (5E11)_CDRL2	GASSRAT	5
DR5 (5E11)_CDRL3	QQGTTHPIT	6
DR5 (18F11)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDAAVYYCAKGVRKKFDYWGQG TLVTVSS	94
DR5 (18F11)_ CDRH1	SYAMS	1
DR5 (18F11)_ CDRH2	AISGSGGSTYYADSVKG	2
DR5 (18F11)_ CDRH3	GVRKKFDY	98
DR5 (18F11)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQ KPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGQLPPITFGQGTKVEIK	95
DR5 (18F11)_ CDRL1	RASQSVSSSYLA	4
DR5 (18F11)_ CDRL2	GASSRAT	5
DR5 (18F11)_ CDRL3	QQGQLPPIT	97

2. Amino acid sequences of non-functional phage display derived DR5 binders

(CDRH1=SEQ ID NO.:1, CDRH2=SEQ ID NO.:2, CDRL1=SEQ ID NO.:4 and CDRL2=SEQ ID NO.:5)

Name	Amino acid sequence	SEQ ID

		NO
DR5 (1B12)_CDR H3	GFGYWYMDY	266
DR5 (1B12)_CDR L3	QQSGRRQT	267
DR5 (19C12)_CDR H3	SIFYSTLDY	268
DR5 (19C12)_CDR L3	QQQGWFQT	269
DR5 (19D6)_CDR H3	VLGYASYDY	270
DR5 (19D6)_CDR L3	QQQGWSTT	271
DR5 (20E3)_CDR H3	GTRRGFDY	272
DR5 (20E3)_CDR L3	QQGELTPVT	273

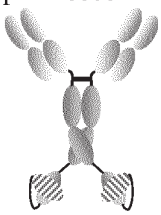
3. Amino acid sequences of FAP binders

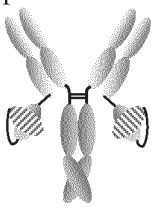
Name	Amino acid sequence	SEQ ID NO
FAP(28H1)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWV RQAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYWGQG TLVTVSS	15
FAP(28H1)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQ KPGQAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLE PEDFAVYYCQQGQVIPPTFGQGTKVEIK	16
FAP(4B9)_VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWV RQAPGKGLEWVSAIIGSGASTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWGQG TLVTVSS	39
FAP(4B9)_VL	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQ KPGQAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRL EPEDFAVYYCQQGIMLPPTFGQGTKVEIK	40
FAP (28H1)_CDRH1	SHAMS	9
FAP (28H1)_CDRH2	AIWASGEQYYADSVKG	10
FAP (28H1)_CDRH3	GWLGNFDY	11
FAP (28H1)_CDRL1	RASQSVSRSYLA	12
FAP (28H1)_CDRL2	GASTRAT	13
FAP (28H1)_CDRL3	QQGQVIPPT	14
FAP (4B9)_CDRH1	SYAMS	33
FAP (4B9)_CDRH2	AIIGSGASTYYADSVKG	34
FAP (4B9)_CDRH3	GWFGGFNY	35

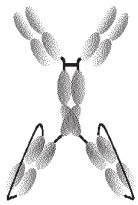
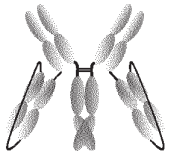
Name	Amino acid sequence	SEQ ID NO
FAP (4B9)_CDRL1	RASQSVTSSYLA	36
FAP (4B9)_CDRL2	VGSRRAT	37
FAP (4B9)_CDRL3	QQGIMLPPT	38

5

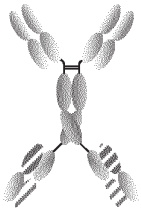
4. Amino acid sequences of bispecific molecules comprising conventional DR5 binders

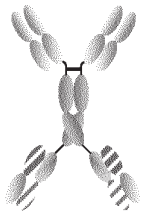
Name	Amino acid sequence	SEQ ID NO
Drozitumab_VH	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SS	274
Drozitumab_VL	SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQA PVLVIYGANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY YCNSADSSGNHVVFGGGTKLTVL	110
Drozitumab-3F2 VHVL-scFv (HC) pETR6606 	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKSGGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKCLEWVSAISGSGGST YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK GWFGGFNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGS EIVLTQSPGTLSLYPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGCGTKVEIK	111

Name	Amino acid sequence	SEQ ID NO
Drozitumab-FAP (4G8) VHVL-scFv (HC) pETR7342	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYW GQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTL SLSPGERATLSCRASQSVSRSYLAWYQQKPGQAPRLLIIGAS TRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQVIP PTFGCGTKVEIK	112
Drozitumab-FAP (4G8) VHVL-scFv (LC) pETR7344 	SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQA PVLVIYGANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY YCNSADSSGNHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVA PTECSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASG FTFSSYAMSWVRQAPGKCLEWVSAISGSGGSTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGT LSLSPGERATLSCRASQSVSRSYLAWYQQKPGQAPRLLIIGA STRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQVI PPTFGCGTKVEIK	113

Name	Amino acid sequence	SEQ ID NO
Drozitumab-3F2 VLCL_VHCH1-scFab (HC) pETR7369 	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLYPGER ATLSCRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSEGG GSEGGGSEGGGSEGGGSGGGSGEVQLLESGGGLVQPGGSLR LSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGW FGGFNYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	114
Drozitumab-3F2 VLCL_VHCH1-scFab (LC) pETR7370 	SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQA PVLVIYGANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY YCNSADSSGNHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVA PTECSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLYPGE RATLSCRASQSVTSSYLAWYQQKPGQAPRLLINVGSRRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGIMLPPTFGQG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSEGG GSEGGGSEGGGSEGGGSGGGSGEVQLLESGGGLVQPGGSLR LSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGW FGGFNYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	115

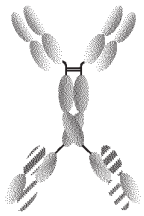
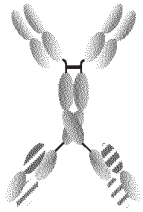
Name	Amino acid sequence	SEQ ID NO
Drozitumab-FAP (4G8) VLCL_VHCH1-scFab (HC) pETR7371	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGER ATLSCRASQSVSRSYLAWYQQKPGQAPRLLIIGASTRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQVIPPTFGQGT KVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSEGG GSEGGGSEGGGSEGGGSGGGSGEVQLLESGGGLVQPGGSLR LSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGW LGNFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	116
Drozitumab-FAP (4G8) VLCL_VHCH1-scFab (LC) pETR7380	SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQA PVLVIYGANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY YCNSADSSGNHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVA PTECSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGE RATLSCRASQSVSRSYLAWYQQKPGQAPRLLIIGASTRATGI PDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQVIPPTFGQG TKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGECSGGGSGGGSEGG GSEGGGSEGGGSEGGGSGGGSGEVQLLESGGGLVQPGGSLR LSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGW LGNFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	117

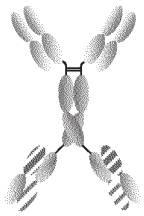
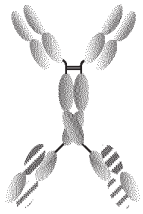
Name	Amino acid sequence	SEQ ID NO
Drozitumab-FAP (4G8) VHCL 2+2 	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKSGGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGST YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK GWLGNFDYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	118
Drozitumab LC pETR7303	SELTQDPAVSVALGQTVRITCSGDSLRSYYASWYQQKPGQA PVLVIYGANNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADY YCNSADSSGNHVVFGGGTKLTVLGQPKAAPSVTLFPPSSEEL QANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSK QSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVA PTECS	119
FAP (4G8) _VLCH1	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CD	120
Drozitumab-FAP (4G8) VLCH1 2+2 	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKSGGGGSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGER ATLSCRASQSVSRSYLAWYQQKPGQAPRLLIIGASTRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQVIPPTFGQGT KVEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVEPKSCD	121

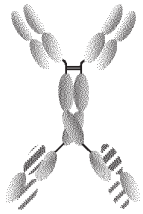
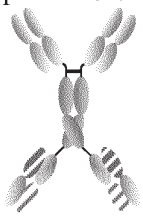
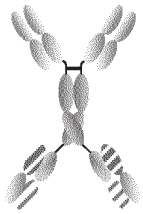
Name	Amino acid sequence	SEQ ID NO
FAP (4G8) _VHCL	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC	122
Drozitumab-FAP (28H1) VHCL pETR9551 2+2 	EVQLVQSGGGVERPGGSLRLSCAASGFTFDDYAMSWVRQA PGKGLEWVSGINWQGGSTGYADSVKGRVTISRDNAKNSLY LQMNSLRAEDTAVYYCAKILGAGRGWYFDYWGKGTTVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKSGGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGS LRLSCAASGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQ YYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK GWLGNFDYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC	123

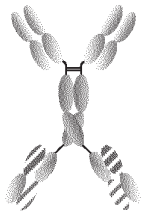
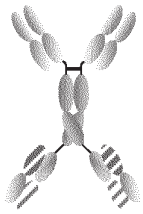
5. Amino acid sequences of bispecific molecules comprising phage display derived DR5 binders

Name	Amino acid sequence	SEQ ID NO
FAP (28H1) _VLCH1 pETR9537	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CD	124

Name	Amino acid sequence	SEQ ID NO
DR5 (22E9)-FAP (28H1) VHCL pETR9711 2+2 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRISFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGG SGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	125
DR5 (22E9) LC pETR9076	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGSNQPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	126
DR5 (21H3)-FAP (28H1) VHCL pETR10626 2+2 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDMAVYYCAKGARVSFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGG SGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	127
DR5 (21H3) LC pETR9075	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGSQPPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	128

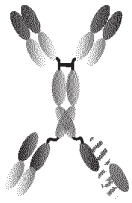
Name	Amino acid sequence	SEQ ID NO
DR5 (20F2)-FAP (28H1) VHCL pETR10135 2+2 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRKGFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGG SGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	129
DR5 (20F2) LC pETR9061	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGESPPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	130
DR5(5E11)-FAP (28H1) VHCL pETR10334 2+2 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGG SGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	131
DR5(5E11) LC pETR9044	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGTTHPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	132

Name	Amino acid sequence	SEQ ID NO
DR5(5E11)-FAP (28H1) VHCL 2+2 Removal of C-term. Lysine in Fc pETR11052 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYW GQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	133
DR5(5E11)-FAP (28H1) VHCL 2+2 Removal of C-term. Lysine in Fc P329G/LALA mut. pETR11025 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL GAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYW GQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	134
VHVL DR5(5E11)-FAP (28H1) pETR11827 2+2 	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGTTHPITFGQGTKVEIKSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGKSGGGGSGGGGSGGGGSGG GGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVR QAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCD	135

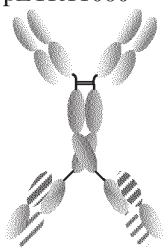
Name	Amino acid sequence	SEQ ID NO
DR5(5E11) VHCL pETR11484	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC	136
FAP (28H1) VLCL pETR9366	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	137
CH1CL DR5(5E11)-FAP (28H1) pETR11828 2+2 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG GGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRL SCAASGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWL GNFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	138
DR5(5E11) VLCH1 pETR11480	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGTTHPITFGQGTKVEIKSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CD	139
DR5(18F11)-FAP (28H1) VHCL 2+2 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDAAVYYCAKGVRKKFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGG SGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	140

Name	Amino acid sequence	SEQ ID NO
DR5(18F11) LC	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQLPPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	141
DR5(5E11)-FAP (28H1) Fc knob VHCL 2+1 pETR10427 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSG GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDYW GQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	142
DR5(5E11) Fc hole pETR10336	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	143
DR5(5E11)-FAP (28H1) Fc knob VHCL 3+1 pETR10427	as above	143

Name	Amino acid sequence	SEQ ID NO
DR5(5E11)-DR5(5E11) Fc hole pETR10429 3+1 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSG GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGVRVSFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCD	144
DR5(5E11)_Fc knob Fab-Fab Head-to-tail 2+1 pETR10662 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCGGGGSGGGGSGEVQLLESGGGLVQPGG SLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGS TYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA KGVRVSFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK	145
FAP (28H1) _Fc hole VHCL pETR10130	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAP GKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSL SCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	146

Name	Amino acid sequence	SEQ ID NO
DR5(18F11)-FAP (28H1) Fc knob VHCL 2+1 pETR9807 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDAAVYYCAKGVRKKFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGG GSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCA ASGFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSV KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNF DYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSS TLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	147
DR5(18F11) Fc hole pETR9808	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDAAVYYCAKGVRKKFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	148
DR5(18F11)-FAP (28H1) VHCL Fc knob 3+1 pETR10333 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDAAVYYCAKGVRKKFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGS GGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAAS GFTFSSHAMSWVRQAPGKGLEWVSAIWASGEQYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWLGNFDY WGQGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNN FYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	149

Name	Amino acid sequence	SEQ ID NO
DR5(18F11)-DR5(18F11) Fc hole pETR10288	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDAAVYYCAKGVRKKFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLSCAVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSG GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGR FTISRDNSKNTLYLQMNSLRAEDAAVYYCAKGVRKKFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCD	150
FAP (28H1) _Fc hole VHCL pETR10130 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAP GKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSL SCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLV SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	159
FAP (28H1) VLCH1 pETR9537	EIVLTQSPGTLSLSPGERATLSCRASQSVSRSYLAWYQQKPG QAPRLLIIGASTRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGQVIPPTFGQGTKVEIKSSASTKGPSVFPLAPSSKSTS GGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CD	160
DR5 (5E11)-Fc knob	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	161
DR5 (5E11)_LC pETR9044	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGTTHPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	162

Name	Amino acid sequence	SEQ ID NO
DR5 (5E11)__ FAP(4B9) VHCL pETR11060 	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLRLSCAASGF TFSSYAMSWVRQAPGKGLEWVSAIIGSGASTYYADSVKGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAKGWFGGFNYWG QGTLVTVSSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	262
FAP(4B9) VLCH1 pETR10020	EIVLTQSPGTLSLSPGERATLSCRASQSVTSSYLAWYQQKPG QAPRLLINVGSRRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQGIMLPPTFGQGTKVEIKSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCD	263
11826 5E11 (VLCL)-Fc-28H1 (VHCH1)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAV YYCQQGTTHPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGECDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQG NVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGG SGGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMS WVRQAPGKGLEWVSAIWASGEQYYADSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTV SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCD	274
11478 5E11 VHCH1	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCD	275

Name	Amino acid sequence	SEQ ID NO
11829 5E11-28H1 VHCH1	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQS VSRSYLAWYQQKPGQAPRLLIIGASTRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQGQVIPPTFGQGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC	276
11830 28H1 VHCH1	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAP GKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCD	277
12207 5E11-28H1 (VLCH1)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAKGVRVSFDYWGQGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL GAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSG GGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERATLSCRASQS VSRSYLAWYQQKPGQAPRLLIIGASTRATGIPDRFSGSGSGT DFTLTISRLEPEDFAVYYCQQGQVIPPTFGQGTKVEIKSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSCD	278
12152 28H1 (VHCL)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVRQAP GKGLEWVSAIWASGEQYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGWLGNFDYWGQGTLVTVSSASVAAP SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC	279

6. Amino acid sequences of Fc domain and constant light chains

Name	Amino acid sequence	SEQ ID NO
hu Fc _wt	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK	151
hu Fc_P329G/LALA	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPK	152
hu kappa light chain	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC	153
hu lambda light chain	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADS SPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTH EGSTVEKTVAPTECS	154

7. Amino acid sequences of DR5 binders derived from rabbit immunization

Clone DR5TAA-0005 (Alias: clone 039)

Heavy chain

VH

qsleesggrlvtpgtpltltctasgfslssaymswvrqapgkglewigyiysgsgstwyaswvkgrftisktsttvdlkitspttedtatyfc argystmgdlwgpgtlvtvss (SEQ ID NO.: 41)

CH1-3

gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntkv dktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvstl piahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaednykt tpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = sayms (SEQ ID NO.: 43)

CDR2 = yiysgsgstwyaswvkg (SEQ ID NO.: 44)

CDR3 = gystmgdl (SEQ ID NO.: 45)

Light Chain

VL

qvltqtpspvsaavggtvtincqasqsvynnrlawyqqkpgqppkllIylastlasgvpsrfkgsgsgtqftltisdlqcddaatyycagg ysgninafgggtevvvk (SEQ ID NO.: 46)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt tsvvqsfnrgdc (SEQ ID NO.: 47)

CDR1 = qasqsvynnrla (SEQ ID NO.: 48)

CDR2 = lastlas (SEQ ID NO.: 49)

CDR3 = aggysgnina (SEQ ID NO.: 50)

Clone DR5TAA-0006 (Alias: clone 058)

Heavy chain

VH

qsleesggrlvtpgtpltltctasgfslssnhmswvrqapgkglewigyiyagsgsayyaswakgrftisrtsttvdlkmtslttedtatyfc agdagssywefnlwgpgtlvtvss (SEQ ID NO.: 51)

CH1-3

gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntkv dktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvstl piahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaednykt tpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = snhms (SEQ ID NO.: 52)

CDR2 = yiyagsgsayyaswakg (SEQ ID NO.: 53)

CDR3 = dagssywefnl (SEQ ID NO.: 54)

Light chain

VL

lvmtqtpsstsepvggtvtikcqasqsigsslswyqqkpgqppkllіyhastlasgvpsrfsgsrsgiqttltisgvqcddaatyyclgvad arrddgfafgggtevvvk (SEQ ID NO.: 55)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt tsvvqsfnrgdc (SEQ ID NO.: 56)

CDR1 = qasqsigssls (SEQ ID NO.: 57)

CDR2 = hastlas (SEQ ID NO.: 58)

CDR3 = lgvadarrddgfa (SEQ ID NO.: 59)

Clone DR5TAA-0010 (Alias: clone 481)

Heavy chain

VH

qsleesggrlvkpdetltltctvsgfslssnaiswvrqapgmglewigiigssgytyyaswakgrftvsktsttvdleiaspttedtatyfcar gysgasdysfnlwgpgtlvtvss (SEQ ID NO.: 60)

CH1-3

gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntkv dktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvstl piahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaednykt tpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = snais (SEQ ID NO.: 61)

CDR2 = iigssgytyyaswakg (SEQ ID NO.: 62)

CDR3 = gysgasdysfnl (SEQ ID NO.: 63)

Light chain

VL

aydmtqtpdsvevavggtvtikcqasqtigdalawyqqkpgqrpnlliyrtstlasgvpsrfsgsgsgthftltisgvecadaatyycqqg atynnvlntfgggtevvvk (SEQ ID NO.: 64)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt tsvvqsfnrgdc (SEQ ID NO.: 47)

CDR1 = qasqtigdala (SEQ ID NO.: 65)

CDR2 = rtstlas (SEQ ID NO.: 66)

CDR3 = qqgatynnvlnt (SEQ ID NO.: 67)

Clone DR5TAA-0013 (Alias: clone 298)

Heavy chain

VH

Qsleesggrlvtpgtpltltctasgftissyhmswvrqapgkglewigyiyagsastwyaswvkgrftisktsttvdlkmtslttedtatyf
cardagssywefnlwgpgtlvtvss (SEQ ID NO.: 68)

CH1-3

gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntkv
dktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvstl
piahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaednykt
tpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = syhms (SEQ ID NO.: 69)

CDR2 = yiyagsastwyaswvkg (SEQ ID NO.: 70)

CDR3 = dagssywefnl (SEQ ID NO.: 54)

Light chain

VL

Lvmtqtpsstsepvggtvtikcqasqsigsslswyqqtpgqppkllliytasslassvpkrfsgsrsgtqftltisgvqcadaatyyclgidd
vrrddgfafgggtevvvk (SEQ ID NO.: 71)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt
tsvvqsfnrgdc (SEQ ID NO.: 47)

CDR1 = qasqsigssls (SEQ ID NO.: 57)

CDR2 = tasslas (SEQ ID NO.: 72)

CDR3 = lgiddvrrddgfa (SEQ ID NO.: 73)

Clone DR5TAA-0019 (Alias: clone 461)

Heavy chain

VH

Qsveesggrlvtpgtpltltctvsgfslsnyamswvrqapgkglewigiisssgttyyaswakgrftisktsttvdlkvtspttedtatyfca
retyygysyaaglwgpgtlvtvss (SEQ ID NO.: 74)

CH1-3

gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntkv
dktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvstl
piahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaednykt
tpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = nyams (SEQ ID NO.: 75)

CDR2 = iisssgttyyaswakg (SEQ ID NO.: 76)

CDR3 = etyygysyaagl (SEQ ID NO.: 77)

Light chain

VL

Alvmtqtpssvsaavggtvtincqasqniysnlawfqqkpgqppkllliyetsklasgvpsrfsgsgsgteftltisdlecddaatyycqss
whsistdcafgggtevvvk (SEQ ID NO.: 78)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt
tsvvqsfnrgdc (SEQ ID NO.: 47)

CDR1 = qasqniysnla (SEQ ID NO.: 79)

CDR2 = etsklas (SEQ ID NO.: 80)

CDR3 = qsswhsistdca (SEQ ID NO.: 81)

Clone DR5TAA-0016 (Alias: clone 422)

Heavy chain

VH

Qsleesggrlvkpdetltltctvsgfslnnyamswvrqapgkglewigminkygtkyyatwtkgratisktsttldleitspttedtatyfc arvryagddyaewldvwgqgilvtvss (SEQ ID NO.: 82)

CH1-3

gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntkv dktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvstl piahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaednykt tpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = nyams (SEQ ID NO.: 75)

CDR2 = minkygtkyyatwtkg (SEQ ID NO.: 83)

CDR3 = vryagddyaewldv (SEQ ID NO.: 84)

Light chain

VL

Adivmtqtaspvsaavggtvtincqasqsisssyvswyqqkpgqppklliykastlasgvpsrfsgsgsgtqlsltirgvqcddaatyyc lygysdvssseyvfgggtevvvr (SEQ ID NO.: 85)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt tsvvqsfnrgdc (SEQ ID NO.: 47)

CDR1 = qasqsisssyvs (SEQ ID NO.: 86)

CDR2 = kastlas (SEQ ID NO.: 28)

CDR3 = lygysdvssseyv (SEQ ID NO.: 87)

Clone DR5TAA-0011 (Alias: clone 174)

Heavy chain

VH

Qsveesggrlvtpgtpltltctvsgfsisryamiwvrqapgegleyigfitsdssayyaswakgrftisktsttvdlkmtspttedtatyfca rytysdgtdlwgpgtlvtvss (SEQ ID NO.: 88)

CH1-3

Gqpkapsvfplapccgdtpsstvtlgclvkgylpepvtvtwnsgtltngvrtfpsvrqssglyslssvvsvtsssqpvtcnvahpatntk vdktvapstcskptcpppellggpsvfifppkpkdtlmisrtpevtcvvvdvsqddpevqftwyinneqvrtarpplreqqfnstirvvs tlpiahqdwlrgkefkckvhnkalpapiektiskargqplepkvytmgppreelssrsvsltcmingfypsdisveweknqkaedny kttpavldsdgsyflynklsvptsewqrgdvftcsvmhealhnhytqksisrspgk (SEQ ID NO.: 42)

CDR1 = ryami (SEQ ID NO.:17)

CDR2 = fitsdssayyaswakg (SEQ ID NO.: 25)

CDR3 = ytysdgtdl (SEQ ID NO.: 19)

Light Chain

VL

Adivmtqtpasvsepvggtvtikcqasqsistylswyqqkpgqppkrliykastlasgvpsrfkgsgsgtdftltirdlecadaatyycq pnsgiatygaafgggtevvvk (SEQ ID NO.: 89)

Ckappa

gdpvaptvlifppaadqvatgtvtivcvankyfpdvtvtwevdgttqttgiensktpqnsadctynlsstltltstqynshkeytckvtqgt tsvvqsfnrgdc (SEQ ID NO.: 47)

CDR1 = qasqsistyls (SEQ ID NO.: 27)

CDR2 = kastlas (SEQ ID NO.:28)

CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

8. Amino acid sequences of chimerized variant of rabbit Mab DR5TAA-0011

Clone DR5TAA-0052 (chimeric variant)

Heavy chain

VH

Qsveesggrlvtpgtpltltctvsgfsisryamiwvrqapgegleyigfitsdssayyaswakgrftisktsttvdlkmtspttedtatyfca rytysdgtdlwgpgtlvtvss (SEQ ID NO.: 90)

CH1-3

Astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps ntkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpr eeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiave wesngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO.: 91)

CDR1 = ryami (SEQ ID NO.: 17)

CDR2 = fitsdssayyaswakg (SEQ ID NO.: 25)

CDR3 = ytysdgtdl (SEQ ID NO.: 19)

Light chain

VH

Adivmtqtpasvsepvggtvtikcqasqsistylswyqqkpgqppkrliykastlasgvpsrfkgsgsgtdftltirdlesadaatyycq pnsgiatygaafgggtevvvk (SEQ ID NO.: 92)

Ckappa

Rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev thqglsspvtksfnrgec (SEQ ID NO.: 93)

CDR1 = qasqsistyls (SEQ ID NO.: 27)

CDR2 = kastlas (SEQ ID NO.: 28)

CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

9. Sequences of humanized variants of rabbit Mab DR5TAA-0011

Variant		HC variant	LC variant
DR5TAA-0066	humanized	VH7	VL3
DR5TAA-0067	humanized	VH7	VL15
DR5TAA-0068	Humanized	VH17	VL10
DR5TAA-0071	Humanized	VH17	VL15
DR5TAA-0072	Humanized	VH17	VL2
DR5TAA-0073	Humanized	VH17	VL3
DR5TAA-0074	Humanized	VH7	VL10
DR5TAA-0075	Humanized	VH7	VL11

Humanized variants Heavy Chain

VH7

VH

Evqlvetgggliqpggslrlscaasgftvsryamiwvrqapgkgleyigfitsdgstyyadsakgrftisrdnsskntlylqmnslraedta vyycarytysdgtdlwgrgtlvtvss (SEQ ID NO.: 23)

CH1-3

astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsn
tkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpre
eqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavew
esngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk constant chain (SEQ
ID NO.: 91)
CDR1 = ryami (SEQ ID NO.: 17)
CDR2 = fitsdgstyyadsakg (SEQ ID NO.: 18)
CDR3 = ytysdgtdl (SEQ ID NO.: 19)

VH17
VH
qvqlvesggglvqpggslrlscsasgfsisryamiwvrqapgkgleyvgfitsdssayyaswakgrftisrdnskntlylqmnslraedt
avyycarytysdgtdlwgqgtlvtvss (SEQ ID NO.: 26)
CH1-3
astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsn
tkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpre
eqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavew
esngqpennykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO.: 91)
CDR1 = ryami (SEQ ID NO.: 17)
CDR2 = fitsdssayyaswakg (SEQ ID NO.: 25)
CDR3 = ytysdgtdl (SEQ ID NO.: 19)

Humanized variants Light Chain

VL2
VL
Diqmtqspstlsasvgdrvtitcrasqsistylswyqqkpgkapkrliykasslasgvpsrfsgsgsgteftltisslqpddaatyycqpns
giatygaafgggtkveik (SEQ ID NO.: 32)
Ckappa
Rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 93)
CDR1 = rasqsistyls (SEQ ID NO.: 20)
CDR2 = kasslas (SEQ ID NO.:21)
CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

VL3
VL
Diqmtqspsslsasvgdrvtitcrasqsistylswyqqkpgkapkrliykastlasgvpsrfsgsgsgtdftltisslqpedaatyycqpns
giatygaafgggtkveik (SEQ ID NO.: 30)
Ckappa
Rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 93)
CDR1 = rasqsistyls (SEQ ID NO.:20)
CDR2 = kastlas (SEQ ID NO.: 28)
CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

VL10
VL
diqmtqspsslsasvgdrvtitcrasqsistylswyqqkpgqppkrliykastlasgvpsrfsgsgsgtdftltisslqpedfatyycqpnsg
iatygaafgggtkveik (SEQ ID NO.: 31)

Ckappa
rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslssttltlskadyekhkvyacevt
hqglsspvtksfnrgec (SEQ ID NO.: 93)
CDR1 = rasqsistyls (SEQ ID NO.:20)
CDR2 = kastlas (SEQ ID NO.: 28)
CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

VL11
VL
diqmtqspsslsasvgdrvtitcqasqsistylswyqqkpgqppkrliykastlasgvpsrfsgsgsgtdftltisslqpedfatyycqpns
giatygaafgggtkveik (SEQ ID NO.: 29)
Ckappa
rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslssttltlskadyekhkvyacevt
hqglsspvtksfnrgec (SEQ ID NO.: 93)
CDR1 = qasqsistyls (SEQ ID NO.: 27)
CDR2 = kastlas (SEQ ID NO.: 28)
CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

VL15
VL
Adiqmtqspstlsasvgdrvtitcrasqsistylswyqqkpgkapkrliykasslasgvpsrfsgsgsgteftltisslqpddaatyycqpn
sgiatygaafgggtkveik (SEQ ID NO.: 24)
Ckappa
Rtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslssttltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 93)
CDR1 = rasqsistyls (SEQ ID NO.: 20)
CDR2 = kasslas (SEQ ID NO.: 21)
CDR3 = qpnsgiatygaa (SEQ ID NO.: 22)

10. Amino acid sequences of chimeric bispecific constructs containing rabbit-derived DR5-binder

Construct DR5TAA-0061 (1+1 chimeric Crossmab containing 4B9 and DR5TAA-0011)

LC (DR5)

adivmtqtpasvsepvggtvtikcqasqsistylswyqqkpgqppkrliykastlasgvpsrfkgsgsgtdftltir
dlecadaatyycqpnsgiatygaafgggtevvvkrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslssttltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 280)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvtssylawyqqkpgqaprllinvgsrratgipdrfsgsgsgtdftltis
rlepedfavyycqqgimlpptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 281)

HC (DR5)

qsveesggrlvtpgtpltltctvsgfsisryamiwvrqapgegleyigfitsdssayyaswakgrftisktsttvdl
kmtspttedtatyfcarytysdgtdlwgpgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvs
wnsgaltsgvhtfpavlqssglylssvvtvpssslgtqtyicnvn
hkpsntkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvd
gvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvctlppsrde
ltknqvslscavkgfypsdiavewesngqpennykttppvldsdgs
fflvskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO.: 282)

Crossed HC (FAP)

evqllesggglvqpggslrlscaasgftfssyamswvrqapgkglewvsaiigsgastyyadsvkgrftisrdnskn
tlylqmnslraedtavyycakgwfggfnywgqgtlvtvssasvaapsvfifppsdeqlksgtasvvcllnnfyprea
kvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhk
vyacevthqglsspvtksfnrgecdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpev
kfnwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvyt
lppcrdeltknqvslwclvkgfypsdiavewesngqpennykttpp
vldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgk (SEQ ID NO.: 283)

Construct DR5TAA-0032 (2+2 chimeric CrossMab containing 28H1 and DR5TAA-0005)

LC (DR5)

aqvltqtpspvsaavggtvtincqasqsvynnrlawyqqkpgqppklliylastlasgvpsrfkgsgsgtqftltis
dlqcddaatyycaggysgninafgggtevvvkrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdn
alqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevth
qglsspvtksfnrgec (SEQ ID NO.: 284)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglylssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 285)

HC (DR5 – crossed FAP)

qsleesggrlvtpgtpltltctasgfslssaymswvrqapgkglewigyiysgsgstwyaswvkgrftisktsttvdlkitspttedtatyfcargystmg
dlwgpgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglylssvvtvpssslgtqtyicnvnh
kpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqyn
styrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsrdeltknqvsltclvkgfypsdiavewesngqpennyktt
ppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgksggggsggggsggggsggggsevqllesggglvqpggslrls
caasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnskntlylqmnslraedtavyycakgwlgnfdywgqgtlvtvs
sasvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglssp
vtksfnrgec
(SEQ ID NO.: 286)

Construct DR5TAA-0033 (2+2 chimeric CrossMab containing 28H1 and DR5TAA-0011)

LC (DR5)

adivmtqtpasvsepvggtvtikcqasqsistylswyqqkpgqppkrliykastlasgvpsrfkgsgsgtdftltir
dlecadaatyycqpnsgiatygaafgggtevvvkrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 287)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 288)

HC (DR5 - crossed FAP)

qsveesggrlvtpgtpltltctvsgfsisryamiwvrqapgegleyigfitsdssayyaswakgrftisktsttvdl
kmtspttedtatyfcarytysdgtdlwgpgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvs
wnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvn
hkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnwyvd
gvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsrde
ltknqvsltclvkgfypsdiavewesngqpennykttppvldsdgs
fflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgksggggsggggsggggsggggsevqllesggglv
qpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnsknt lylqmnslraed
tavyycakgwlgnfdywgqgtlvtvssasvaapsvfifppsdeqlk
sgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqglss
pvtksfnrgec (SEQ ID NO.: 289)

Construct DR5TAA-0034 (2+2 chimeric CrossMab containing 28H1 and DR5TAA-0013)

LC (DR5)

alvmtqtpsstsepvggtvtikcqasqsigsslswyqqtpgqppkllıytasslassvpkrfsgsrsgtqftltisg
vqcadaatyyclgiddvrrddgfafgggtevvvkrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 290)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 291)

HC (DR5 - crossed FAP)

qsleesggrlvtpgtpltltctasgftissyhmswvrqapgkglewigyiyagsastwyaswvkgrftisktsttvd
lkmtslttedtatyfcardagssywefnlwgpgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepv
tvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyic
nvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlpps
rdeltknqvsltclvkgfypsdiavewesngqpennykttppvlds
dgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgksggggsggggsggggsggggsevqllesgg
glvqpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnsknt lylqmnslr
aedtavyycakgwlgnfdywgqgtlvtvssasvaapsvfifppsde
qlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqg
lsspvtksfnrgec (SEQ ID NO.: 292)

Construct DR5TAA-0035 (2+2 chimeric CrossMab containing 28H1 and DR5TAA-0016)

LC (DR5)

adivmtqtaspvsaavggtvtincqasqsisssyvswyqqkpgqppkllliykastlasgvpsrfsgsgsgtqlslti
rgvqcddaatyyclygysdvssseyvfgggtevvvrrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqw
kvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyac
evthqglsspvtksfnrgec (SEQ ID NO.: 293)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 294)

HC (DR5 - crossed FAP)

qsleesggrlvkpdetltltctvsgfslnnyamswvrqapgkglewigminkygtkyyatwtkgratisktsttldl
eitspttedtatyfcarvryagddyaewldvwgqgilvtvssastkgpsvfplapsskstsggtaalgclvkdyfpe
pvtvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqty
icnvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlp
psrdeltknqvsltclvkgfypsdiavewesngqpennykttppvl
dsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgksggggsggggsggggsggggsevqlles
ggglvqpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnskntlylqmns
lraedtavyycakgwlgnfdywgqgtlvtvssasvaapsvfifpps
deqlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevth
qglsspvtksfnrgec (SEQ ID NO.: 295)

Construct DR5TAA-0036 (2+2 chimeric CrossMab containing 28H1 and DR5TAA-0019)

LC (DR5)

alvmtqtpssvsaavggtvtincqasqniysnlawfqqkpgqppkllliyetsklasgvpsrfsgsgsgteftltisd
lecddaatyycqsswhsistdcafgggtevvvkrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvd
nalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevt
hqglsspvtksfnrgec (SEQ ID NO.: 296)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 297)

HC (DR5 - crossed FAP)

qsveesggrlvtpgtpltltctvsgfslsnyamswvrqapgkglewigiisssgttyyaswakgrftisktsttvdl
kvtspttedtatyfcaretyygysyaaglwgpgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepv
tvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyic
nvnhkpsntkvdkkvepkscdkthtcppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlpps
rdeltknqvsltclvkgfypsdiavewesngqpennykttppvlds
dgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgksggggsggggsggggsggggsevqllesgg
glvqpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnskntlylqmnslr
aedtavyycakgwlgnfdywgqgtlvtvssasvaapsvfifppsde
qlksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqg
lsspvtksfnrgec (SEQ ID NO.: 298)

11. Amino acid sequences of bispecific constructs containing humanized rabbit-derived DR5-binder

Construct DR5TAA-0117 (2+2 humanized CrossMab containing 28H1 and DR5TAA-0067)

LC (DR5)

adiqmtqspstlsasvgdrvtitcrasqsistylswyqqkpgkapkrliykasslasgvpsrfsgsgsgteftltis
slqpddaatyycqpnsgiatygaafgggtkveikrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 299)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 300)

HC (DR5 – crossed FAP)

evqlvetgggliqpggslrlscaasgftvsryamiwvrqapgkgleyigfitsdgstyyadsakgrftisrdnsknt
lylqmnslraedtavyycarytysdgtdlwgrgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepv
tvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyic
nvnhkpsntkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlpps
rdeltknqvsltclvkgfypsdiavewesngqpennykttppvlds
dgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgggggsggggsggggsggggsevqllesgggl
vqpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnsknt lylqmnslrae
dtavyycakgwlgnfdywgqgtlvtvssasvaapsvfifppsdeql
ksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqgls
spvtksfnrgec (SEQ ID NO.: 301)

Construct DR5TAA-0118 (2+2 humanized CrossMab containing 28H1 and DR5TAA-0071)

LC (DR5)

adiqmtqspstlsasvgdrvtitcrasqsistylswyqqkpgkapkrliykasslasgvpsrfsgsgsgteftltis
slqpddaatyycqpnsgiatygaafgggtkveikrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkv
dnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacev
thqglsspvtksfnrgec (SEQ ID NO.: 302)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 303)

HC (DR5 – crossed FAP)

qvqlvesggglvqpggslrlscsasgfsisryamiwvrqapgkgleyvgfitsdssayyaswakgrftisrdnsknt
lylqmnslraedtavyycarytysdgtdlwgqgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepv
tvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyic
nvnhkpsntkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlpps
rdeltknqvsltclvkgfypsdiavewesngqpennykttppvlds

dgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgggggsggggsggggsggggsevqllesgggl
vqpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnskntlylqmnslrae
dtavyycakgwlgnfdywgqgtlvtvssasvaapsvfifppsdeql
ksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqgls
spvtksfnrgec (SEQ ID NO.: 304)

Construct DR5TAA-0119 (2+2 humanized CrossMab containing 28H1 and DR5TAA-0075)

LC (DR5)

diqmtqspsslsasvgdrvtitcqasqsistylswyqqkpgqppkrliykastlasgvpsrfsgsgsgtdftltiss
lqpedfatyycqpnsgiatygaafgggtkveikrtvaapsvfifppsdeqlksgtasvvcllnnfypreakvqwkvd
nalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevt
hqglsspvtksfnrgec (SEQ ID NO.: 305)

Crossed LC (FAP)

eivltqspgtlslspgeratlscrasqsvsrsylawyqqkpgqaprlliigastratgipdrfsgsgsgtdftltis
rlepedfavyycqqgqvipptfgqgtkveikssastkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsg
altsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkps
ntkvdkkvepkscd (SEQ ID NO.: 306)

HC (DR5 – crossed FAP)

evqlvetgggliqpggslrlscaasgftvsryamiwvrqapgkgleyigfitsdgstyyadsakgrftisrdnsknt
lylqmnslraedtavyycarytysdgtdlwgrgtlvtvssastkgpsvfplapsskstsggtaalgclvkdyfpepv
tvswnsgaltsgvhtfpavlqssglyslssvvtvpssslgtqtyic
nvnhkpsntkvdkkvepkscdkthtcppcpapeaaggpsvflfppkpkdtlmisrtpevtcvvvdvshedpevkfnw
yvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykckvsnkalgapiektiskakgqprepqvytlpps
rdeltknqvsltclvkgfypsdiavewesngqpennykttppvlds
dgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslslspgggggsggggsggggsggggsevqllesgggl
vqpggslrlscaasgftfsshamswvrqapgkglewvsaiwasgeqyyadsvkgrftisrdnskntlylqmnslrae
dtavyycakgwlgnfdywgqgtlvtvssasvaapsvfifppsdeql
ksgtasvvcllnnfypreakvqwkvdnalqsgnsqesvteqdskdstyslsstltlskadyekhkvyacevthqgls
spvtksfnrgec (SEQ ID NO.: 307)

12. Amino acid sequences of C-terminal fusions of humanized rabbit-derived DR5-binder

Description	Amino acid sequences	SEQ ID NO
huFc-hVH007	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGG SGGGGSEVQLVETGGGLIQPGGSLRLSCAASGFTVSRYAMIWVRQAPG KGLEYIGFITSDGSTYYADSAKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYTYSDGTDLWGRGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	308
hVL015	DIQMTQSPSTLSASVGDRVTITCRASQSISTYLSWYQQKPGKAPKRLI YKASSLASGVPSRFSGSGSGTEFTLTISSLQPDDAATYYCQPNSGIAT YGAAFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP	309

	REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	
huFc-hVH017	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGGGGSGGGG SGGGGSQVQLVESGGGLVQPGGSLRLSCSASGFSISRYAMIWVRQAPG KGLEYVGFITSDSSAYYASWAKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCARYTYSDGTDLWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD	310
hVL011	DIQMTQSPSSLSASVGDRVTITCQASQSISTYLSWYQQKPGQPPKRLI YKASTLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQPNSGIAT YGAAFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC	311

13. Nucleic acid sequences of DR5 binders and bispecific molecules comprising them

Description	Nucleotide sequences	SEQ ID NO
DR5 (22E9)_VH	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGTGTGCGGATTTCGTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGT	167
DR5(22E9)_CDRH1	AGTTATGCCATGAGC	168
DR5 (22E9)_CDRH2	GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGC	169
DR5 (22E9)_CDRH3	GGTGTGCGGATTTCGTTTGACTAC	170
DR5 (22E9)_VL	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTTCTAATCAGCCCGT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAA	171
DR5 (22E9)_CDRL1	AGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCC	172
DR5	GGAGCATCCAGCAGGGCCACT	173

Description	Nucleotide sequences	SEQ ID NO
(22E9)_CDRL2		
DR5 (22E9)_CDRL3	CAGCAGGGTTCTAATCAGCCCGTTACG	174
DR5 (21H3)_VH	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACATGGCCGTATATTACTGTG CGAAAGGTGCTCGTGTTTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGT	175
DR5 (21H3)_CDRH1	AGTTATGCCATGAGC	176
DR5 (21H3)_CDRH2	GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGC	177
DR5 (21H3)_CDRH3	GGTGCTCGTGTTTCTTTTGACTAC	178
DR5 (21H3)_VL	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCGGTGTATTACTGTCAGCAGGGTTCTCAGCCGCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAA	179
DR5 (21H3)_CDRL1	AGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCC	180
DR5 (21H3)_CDRL2	GGAGCATCCAGCAGGGCCACT	181
DR5 (21H3)_CDRL3	CAGCAGGGTTCTCAGCCGCCCATTACG	182
DR5 (20F2)_VH	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGTGTGAGGAAGGGGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGT	183
DR5 (20F2)_CDRH1	AGTTATGCCATGAGC	184
DR5 (20F2)_CD	GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGC	185

Description	Nucleotide sequences	SEQ ID NO
RH2		
DR5 (20F2)_CD RH3	GGTGTGAGGAAGGGGTTTGACTAC	186
DR5 (20F2)_VL	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTGAGTCGCCTCCCCC GACGTTCGGCCAGGGGACCAAAGTGGAAATCAAA	187
DR5 (20F2)_CD RL1	AGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCC	188
DR5 (20F2)_CD RL2	GGAGCATCCAGCAGGGCCACT	189
DR5 (20F2)_CD RL3	CAGCAGGGTGAGTCGCCTCCCCCGACG	190
DR5(5E11) _VH	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGT	191
DR5(5E11) _CDRH1	AGTTATGCCATGAGC	192
DR5(5E11) _CDRH2	GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGC	193
DR5(5E11) _CDRH3	GGGGTGAGGGTGTCTTTTGACTAC	194
DR5(5E11) _VL	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTACTACTCATCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAA	195
DR5(5E11) _CDRL1	AGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCC	196
DR5(5E11) _CDRL2	GGAGCATCCAGCAGGGCCACT	197
DR5(5E11) _CDRL3	CAGCAGGGTACTACTCATCCCATTACG	198
DR5(18F11)_VH	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA	199

Description	Nucleotide sequences	SEQ ID NO
	AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGTATATTACTGTG CGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGT	
DR5(18F11)_CDRH1	AGTTATGCCATGAGC	200
DR5(18F11)_CDRH2	GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAG GGC	201
DR5(18F11)_CDRH3	GGGGTGCGTAAGAAGTTTGACTAC	202
DR5(18F11)_VL	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTCAGTTGCCTCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAA	203
DR5(18F11)_CDRL1	AGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCC	204
DR5(18F11)_CDRL2	GGAGCATCCAGCAGGGCCACT	205
DR5(18F11)_CDRL3	CAGCAGGGTCAGTTGCCTCCCATTACG	206
FAP (28H1)_V H	GAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGG ATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCTCCTCCCAC GCCATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCTGGAATGGGTG TCCGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGACTCTGTGAAG GGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTACCTG CAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCC AAGGGCTGGCTGGGCAACTTCGACTACTGGGGACAGGGCACCCTGGTC ACCGTGTCCAGC	207
FAP (28H1)__V L	GAGATCGTGCTGACCCAGTCCCCCGGCACCCTGTCTCTGAGCCCTGGC GAGAGAGCCACCCTGTCCTGCAGAGCCTCCCAGTCCGTGTCCCGGTCC TACCTCGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCTCGGCTGCTG ATCATCGGCGCCTCTACCAGAGCCACCGGCATCCCTGACCGGTTCTCC GGCTCTGGCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTGGAA CCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCCAGGTCATCCCT CCCACCTTTGGCCAGGGCACCAAGGTGGAAATCAAG	208
FAP (4B9)__VH	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CTATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTATTGGTAGTGGTGCTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGTGGTTTGGTGGTTTTAACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGT	209
FAP (4B9)__VL	GAGATCGTGCTGACCCAGTCCCCCGGCACCCTGTCTCTGAGCCCTGGC GAGAGAGCCACCCTGTCCTGCAGAGCCTCCCAGTCCGTGACCTCCTCC TACCTCGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCTCGGCTGCTG ATCAACGTGGGCAGTCGGAGAGCCACCGGCATCCCTGACCGGTTCTCC GGCTCTGGCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTGGAA	210

Description	Nucleotide sequences	SEQ ID NO
	CCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCATCATGCTGCCC CCCACCTTTGGCCAGGGCACCAAGGTGGAAATCAAG	
Drozitumab_VH	GAAGTGCAGCTGGTGCAGTCTGGCGGCGGAGTGGAAAGACCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGTCTTGGGTCCGCCAGGCCCCTGGAAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGCGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGAGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACTGTGTCT AGC	211
Drozitumab_VL	AGCGAGCTGACCCAGGATCCTGCCGTGTCTGTGGCTCTGGGCCAGACC GTGCGGATCACCTGTAGCGGCGACAGCCTGCGGAGCTACTACGCCAGC TGGTATCAGCAGAAGCCCGGCCAGGCTCCCGTGCTGGTGATCTACGGC GCCAACAACAGACCCAGCGGCATCCCCGACAGATTCAGCGGCAGCAG CAGCGGCAATACCGCCAGCCTGACCATCACAGGCGCCCAGGCCGAGG ACGAGGCCGACTACTACTGCAACAGCGCCGACAGCTCCGGCAACCAC GTGGTGTTCGGCGGAGGCACCAAGCTGACCGTCCTAGGT	212
Drozitumab – 3F2 scFv (HC) pETR6606	GAGGTGCAGCTGGTGCAGAGCGGCGGAGGGGTGGAGAGGCCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGAGCTGGGTGCGCCAGGCCCCTGGCAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGAGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGCGGGCCGAGGACACAGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGGGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACCGTGTCCAGCGCTAGCACCAAGGGCCC ATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAGCTG CAGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC ACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGC CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGC TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTAAATCCGGAGGCGGAGGAAGTGGAGGGGGAGGA TCCGGAGGGGGCGGATCTGGCGGCGGAGGCAGCGAGGTGCAATTGCT GGAAAGCGGAGGCGGACTCGTGCAGCCTGGCGGCAGCCTGAGACTGA GCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGTCTTGGG TCCGGCAGGCCCCTGGAAAGTGCCTGGAATGGGTGTCCGCCATCAGCG	213

Description	Nucleotide sequences	SEQ ID NO
	GCAGCGGCGGCAGCACCTACTACGCCGACAGCGTGAAGGGCCGGTTC ACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTCCAGATGAA CAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCCAAGGGAT GGTTCGGCGGCTTCAACTACTGGGGCCAGGGCACCCTGGTCACAGTCT CGAGTGGCGGAGGGGGATCTGGGGGAGGCGGATCAGGAGGAGGAGG AAGCGGGGGAGGGGGCAGCGAGATCGTGTTAACGCAGAGCCCCGGCA CCCTGAGCCTGTATCCCGGCGAGAGAGCCACCCTGAGCTGCAGAGCCA GCCAGAGCGTGACCAGCAGCTACCTGGCCTGGTATCAGCAGAAGCCCG GCCAGGCCCCCAGACTGCTGATCAACGTGGGCAGCAGAAGGGCCACC GGCATCCCCGACAGATTCAGCGGCTCCGGCAGCGGCACCGACTTCACC CTGACCATCAGCAGACTGGAACCCGAGGATTTCGCCGTGTATTATTGC CAGCAGGGCATCATGCTGCCCCCTACCTTCGGATGCGGCACCAAGGTG GAGATCAAG	
Drozitumab-FAP (4G8) scFv (HC) pETR7342	GAGGTGCAGCTGGTGCAGAGCGGCGGAGGGGTGGAGAGGCCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGAGCTGGGTGCGCCAGGCCCCTGGCAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGAGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGCGGGCCGAGGACACAGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGGGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACCGTGTCCAGCGCTAGCACCAAGGGCCC ATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCC TGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC ACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGC CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGC TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTGGCGGGTCCGGAGGCGGAGGAAGTGGCGGCGGA GGCAGCGAAGTGCAGCTGCTGGAAAGCGGCGGAGGACTGGTGCAGCC TGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAG CAGCTACGCCATGTCTTGGGTCCGCCAGGCCCCTGGAAAGTGCCTGGA ATGGGTGTCCGCCATCAGCGGCAGCGGCGGCAGCACCTACTACGCCGA CAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACA CCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGT ACTACTGCGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGCCAGG GCACTCTGGTCACAGTGTCTAGCGGAGGCGGCGGATCTGGCGGAGGTG GAAGCGGAGGGGGAGGATCAGGGGGCGGAGGCTCCGAGATCGTGCTG	214

Description	Nucleotide sequences	SEQ ID NO
	ACCCAGAGCCCTGGCACACTGTCTCTGAGCCCTGGCGAGAGAGCCACC CTGAGCTGCAGAGCCAGCCAGAGCGTGTCCAGAAGCTACCTGGCTTGG TATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCTGATCATCGGCGCT AGCACCAGAGCCACCGGCATTCCCGACAGATTCAGCGGCTCCGGCAGC GGCACCGACTTCACCCTGACCATCAGCAGACTGGAACCCGAGGATTTC GCCGTCTATTATTGCCAGCAGGGCCAAGTCATCCCTCCTACCTTCGGAT GCGGCACTAAGGTGGAGATCAAG	
Drozitumab -FAP (4G8) scFv (LC) pETR7344	AGCGAGCTGACCCAGGACCCCGCCGTGAGCGTGGCCCTGGGACAGAC CGTGCGGATCACCTGCAGCGGCGACAGCCTGCGCAGCTACTACGCCAG CTGGTATCAGCAGAAGCCCGGCCAGGCCCCCGTGCTGGTGATCTACGG CGCCAACAACCGGCCCAGCGGCATCCCCGACCGGTTCAGCGGCAGCA GCAGCGGCAACACCGCCAGCCTGACCATCACAGGCGCCCAGGCCGAG GACGAGGCCGACTACTACTGCAACAGCGCCGACAGCTCCGGCAACCA CGTGGTGTTTGGCGGCGGAACAAAGCTGACCGTCCTAGGTCAACCCAA GGCTGCCCCCAGCGTGACCCTGTTCCCCCCCCAGCAGCGAGGAACTGCA GGCCAACAAGGCCACCCTGGTCTGCCTGATCAGCGACTTCTACCCAGG CGCCGTGACCGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCG GCGTGGAGACCACCACCCCCAGCAAGCAGAGCAACAACAAGTACGCC GCCAGCAGCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCACAG GTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCACCGTGGAGAAAA CCGTGGCCCCCACCGAGTGCTCCGGAGGCGGAGGAAGTGGCGGCGGA GGCAGCGAAGTGCAGCTGCTGGAAAGCGGCGGAGGACTGGTGCAGCC TGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCAG CAGCTACGCCATGTCTTGGGTCCGCCAGGCCCCTGGAAAGTGCCTGGA ATGGGTGTCCGCCATCAGCGGCAGCGGCGGCAGCACCTACTACGCCGA CAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACA CCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGT ACTACTGCGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGCCAGG GCACTCTGGTCACAGTGTCTAGCGGAGGCGGCGGATCTGGCGGAGGTG GAAGCGGAGGGGGAGGATCAGGGGGCGGAGGCTCCGAGATCGTGCTG ACCCAGAGCCCTGGCACACTGTCTCTGAGCCCTGGCGAGAGAGCCACC CTGAGCTGCAGAGCCAGCCAGAGCGTGTCCAGAAGCTACCTGGCTTGG TATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCTGATCATCGGCGCT AGCACCAGAGCCACCGGCATTCCCGACAGATTCAGCGGCTCCGGCAGC GGCACCGACTTCACCCTGACCATCAGCAGACTGGAACCCGAGGATTTC GCCGTCTATTATTGCCAGCAGGGCCAAGTCATCCCTCCTACCTTCGGAT GCGGCACTAAGGTGGAGATCAAG	215
Drozitumab – 3F2 scFab (HC) pETR7369	GAGGTGCAGCTGGTGCAGAGCGGCGGAGGGGTGGAGAGGCCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGAGCTGGGTGCGCCAGGCCCCTGGCAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGAGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGCGGGCCGAGGACACAGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGGGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACCGTGTCCAGCGCTAGCACCAAGGGCCC ATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT	216

Description	Nucleotide sequences	SEQ ID NO
	CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCC TGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC ACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGC CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGC TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTAAATCCGGAGGCGGAGGTTCCGGTGGAGGCGGAT CCGGAGGAGGTGGCAGCGGAGGTGGTGGCTCCGAAATCGTGTTAACG CAGTCTCCAGGCACCCTGTCTTTGTATCCAGGGGAAAGAGCCACCCTC TCTTGCAGGGCCAGTCAGAGTGTTACCAGTAGCTACTTAGCCTGGTAC CAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCTCC CGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGATCCGGG ACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCA GTGTATTACTGTCAGCAGGGTATTATGCTTCCCCCGACGTTCGGCCAGG GGACCAAAGTGGAAATCAAACGTACGGTGGCCGCTCCCAGCGTGTTCA TCTTCCCCCCCAGCGACGAGCAGCTGAAGTCCGGCACCGCCAGCGTGG TGTGCCTGCTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGA AGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACC GAGCAGGACAGCAAGGACTCCACCTACAGCCTGTCCAGCACCCTGACC CTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGT GACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAGCTTCAACCGGG GCGAGTGCTCCGGCGGAGGATCTGGGGGAGGAAGCGAAGGAGGCGGA TCTGAGGGCGGTGGCTCTGAAGGCGGTGGAAGTGAGGGAGGCGGTAG CGGAGGTGGATCCGGCGAGGTGCAATTGTTGGAGTCTGGGGGAGGCTT GGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGATT CACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAA GGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATA CTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTC CAAGAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGACA CGGCCGTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTACTG GGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTAGCACAAAGGGCCC CAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGAAC AGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTGAC TGTGTCCTGGAACAGCGGTGCTCTCACATCTGGGGTCCACACCTTTCCA GCCGTGCTCCAGTCCTCAGGGCTCTACAGCCTGAGCAGCGTCGTCACA GTCCCATCTAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAAC CACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGAG CTGTGAC	
Drozitumab-3F2 scFab (LC) pETR7370	AGCGAGCTGACCCAGGACCCCGCCGTGAGCGTGGCCCTGGGACAGAC CGTGCGGATCACCTGCAGCGGCGACAGCCTGCGCAGCTACTACGCCAG CTGGTATCAGCAGAAGCCCGGCCAGGCCCCCGTGCTGGTGATCTACGG CGCCAACAACCGGCCCAGCGGCATCCCCGACCGGTTCAGCGGCAGCA GCAGCGGCAACACCGCCAGCCTGACCATCACAGGCGCCCAGGCCGAG	217

Description	Nucleotide sequences	SEQ ID NO
	GACGAGGCCGACTACTACTGCAACAGCGCCGACAGCTCCGGCAACCA CGTGGTGTTTGGCGGCGGAACAAAGCTGACCGTCCTAGGTCAACCCAA GGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAACTGCA GGCCAACAAGGCCACCCTGGTCTGCCTGATCAGCGACTTCTACCCAGG CGCCGTGACCGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCG GCGTGGAGACCACCACCCCCAGCAAGCAGAGCAACAACAAGTACGCC GCCAGCAGCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCACAG GTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCACCGTGGAGAAAA CCGTGGCCCCCACCGAGTGCTCCGGAGGCGGAGGTTCCGGTGGAGGCG GATCCGGAGGAGGTGGCAGCGGAGGTGGTGGCTCCGAAATCGTGTTA ACGCAGTCTCCAGGCACCCTGTCTTTGTATCCAGGGGAAAGAGCCACC CTCTCTTGCAGGGCCAGTCAGAGTGTTACCAGTAGCTACTTAGCCTGGT ACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCAATGTGGGCT CCCGTAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGATCCG GGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTG CAGTGTATTACTGTCAGCAGGGTATTATGCTTCCCCCGACGTTCGGCCA GGGGACCAAAGTGGAAATCAAACGTACGGTGGCCGCTCCCAGCGTGTT CATCTTCCCCCCCAGCGACGAGCAGCTGAAGTCCGGCACCGCCAGCGT GGTGTGCCTGCTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTG GAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCA CCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGTCCAGCACCCTGA CCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAA GTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAGCTTCAACCGG GGCGAGTGCTCCGGCGGAGGATCTGGGGGAGGAAGCGAAGGAGGCGG ATCTGAGGGCGGTGGCTCTGAAGGCGGTGGAAGTGAGGGAGGCGGTA GCGGAGGTGGATCCGGCGAGGTGCAATTGTTGGAGTCTGGGGGAGGCT TGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGAT TCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGA AGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACAT ACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATT CCAAGAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGAC ACGGCCGTATATTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTACT GGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTAGCACAAAGGGC CCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGA ACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTG ACTGTGTCCTGGAACAGCGGTGCTCTCACATCTGGGGTCCACACCTTTC CAGCCGTGCTCCAGTCCTCAGGGCTCTACAGCCTGAGCAGCGTCGTCA CAGTCCCATCTAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGA ACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAG AGCTGTGAC	
Drozitumab-FAP (4G8) scFab (HC) pETR7371	GAGGTGCAGCTGGTGCAGAGCGGCGGAGGGGTGGAGAGGCCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGAGCTGGGTGCGCCAGGCCCCTGGCAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGAGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGCGGGCCGAGGACACAGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGGGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACCGTGTCCAGCGCTAGCACCAAGGGCCC ATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCC	218

Description	Nucleotide sequences	SEQ ID NO
	GGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGAC CGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAAT CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCC TGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCC TCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG AGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGC ACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGC CCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACC AGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC ACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGC TCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTAAATCCGGAGGCGGAGGAAGCGGAGGGGGAGGA TCAGGCGGCGGTGGATCAGGCGGTGGAGGATCCGAGATCGTGCTGACC CAGTCCCCTGGCACCCTGTCTCTGAGCCCAGGCGAGAGAGCCACCCTG AGCTGCAGAGCCAGCCAGAGCGTGTCCAGAAGCTATCTGGCTTGGTAT CAGCAGAAGCCCGGCCAGGCCCCCAGACTGCTGATCATCGGCGCCAGC ACCAGAGCCACCGGCATCCCCGACAGATTCAGCGGCAGCGGCTCCGGC ACCGACTTCACCCTGACCATCTCCCGGCTGGAACCCGAGGACTTCGCC GTGTACTACTGCCAGCAGGGCCAGGTCATCCCTCCTACCTTCGGCCAG GGCACCAAGGTGGAGATCAAGCGTACGGTGGCCGCTCCCAGCGTGTTC ATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGTG GTCTGCCTGCTGAACAACTTCTACCCCCGGGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCAC CGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACACTGA CCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAA GTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGG GGCGAGTGTTCTGGTGGCGGATCTGGCGGAGGCAGTGAAGGCGGCGG AAGTGAGGGTGGAGGCAGCGAGGGGGGAGGCTCTGAAGGGGGAGGA AGTGGAGGCGGTTCAGGGGAAGTGCAATTGTTGGAGTCTGGGGGAGG CTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGG ATTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGG GAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCAC ATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAA TTCCAAGAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGA CACGGCCGTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTGACTA CTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTAGCACAAAGGG CCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGG AACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGT GACTGTGTCCTGGAACAGCGGTGCTCTCACATCTGGGGTCCACACCTTT CCAGCCGTGCTCCAGTCCTCAGGGCTCTACAGCCTGAGCAGCGTCGTC ACAGTCCCATCTAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTG AACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAA GAGCTGTGAC	
Drozitumab-FAP (4G8)	AGCGAGCTGACCCAGGACCCCGCCGTGAGCGTGGCCCTGGGACAGAC CGTGCGGATCACCTGCAGCGGCGACAGCCTGCGCAGCTACTACGCCAG	219

Description	Nucleotide sequences	SEQ ID NO
scFab (LC) pETR7380	CTGGTATCAGCAGAAGCCCGGCCAGGCCCCCGTGCTGGTGATCTACGG CGCCAACAACCGGCCCAGCGGCATCCCCGACCGGTTCAGCGGCAGCA GCAGCGGCAACACCGCCAGCCTGACCATCACAGGCGCCCAGGCCGAG GACGAGGCCGACTACTACTGCAACAGCGCCGACAGCTCCGGCAACCA CGTGGTGTTTGGCGGCGGAACAAAGCTGACCGTCCTAGGTCAACCCAA GGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAACTGCA GGCCAACAAGGCCACCCTGGTCTGCCTGATCAGCGACTTCTACCCAGG CGCCGTGACCGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCG GCGTGGAGACCACCACCCCCAGCAAGCAGAGCAACAACAAGTACGCC GCCAGCAGCTACCTGAGCCTGACCCCCGAGCAGTGGAAGAGCCACAG GTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCACCGTGGAGAAAA CCGTGGCCCCCACCGAGTGCTCCGGAGGCGGAGGAAGCGGAGGGGGA GGATCAGGCGGCGGTGGATCAGGCGGTGGAGGATCCGAGATCGTGCT GACCCAGTCCCCTGGCACCCTGTCTCTGAGCCCAGGCGAGAGAGCCAC CCTGAGCTGCAGAGCCAGCCAGAGCGTGTCCAGAAGCTATCTGGCTTG GTATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCTGATCATCGGCGC CAGCACCAGAGCCACCGGCATCCCCGACAGATTCAGCGGCAGCGGCTC CGGCACCGACTTCACCCTGACCATCTCCCGGCTGGAACCCGAGGACTT CGCCGTGTACTACTGCCAGCAGGGCCAGGTCATCCCTCCTACCTTCGG CCAGGGCACCAAGGTGGAGATCAAGCGTACGGTGGCCGCTCCCAGCG TGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCA GCGTGGTCTGCCTGCTGAACAACTTCTACCCCCGGGAGGCCAAGGTGC AGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAAAGC GTCACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCAC ACTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCT GCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCA ACCGGGGCGAGTGTTCTGGTGGCGGATCTGGCGGAGGCAGTGAAGGC GGCGGAAGTGAGGGTGGAGGCAGCGAGGGGGGAGGCTCTGAAGGGG GAGGAAGTGGAGGCGGTTCAGGGGAAGTGCAATTGTTGGAGTCTGGG GGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCC TCCGGATTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTC CAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTA GCACATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAG ACAATTCCAAGAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCG AGGACACGGCCGTATATTACTGTGCGAAAGGGTGGCTGGGTAATTTTG ACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTAGCACAA AGGGCCCCAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCG GCGGAACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGC CCGTGACTGTGTCCTGGAACAGCGGTGCTCTCACATCTGGGGTCCACA CCTTTCCAGCCGTGCTCCAGTCCTCAGGGCTCTACAGCCTGAGCAGCGT CGTCACAGTCCCATCTAGCAGCCTGGGCACCCAGACCTACATCTGCAA CGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGC CCAAGAGCTGTGAC	
Drozitumab -FAP (4G8) VHCL 2+2	GAAGTGCAGCTGGTGCAGTCTGGCGGCGGAGTGGAAAGACCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGTCTTGGGTCCGCCAGGCCCCTGGAAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGCGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGAGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACTGTGTCTAGCGCTAGCACCAAGGGCCCA	220

Description	Nucleotide sequences	SEQ ID NO
	AGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGAAC AGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTGAC AGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTTCC AGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTCAC AGTGCCTAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGA GCTGCGACAAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCT GGGCGGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCT GATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTC CCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGA GGTGCACAATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCA CCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCC CCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCC CAGGTGTACACCCTGCCCCCCAGCAGAGATGAGCTGACCAAGAACCA GGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCAGCGATATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCAC CCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACTCCAAACTG ACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAG CGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAG CCTGAGCCCCGGCAAGTCCGGAGGCGGCGGAAGCGGAGGAGGAGGAT CCGGAGGAGGGGGAAGTGGCGGCGGAGGATCTGAGGTGCAGCTGCTG GAATCTGGAGGCGGCCTGGTGCAGCCTGGCGGCAGCCTGAGACTGTCT TGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAGCTGGGTC CGACAGGCTCCTGGCAAGGGACTGGAATGGGTGTCCGCCATCTCCGGC AGCGGAGGCAGCACCTACTACGCCGACAGCGTGAAGGGCCGGTTCAC CATCAGCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAACA GCCTGCGGGCCGAGGATACCGCCGTGTATTATTGCGCCAAGGGATGGC TGGGCAACTTCGACTACTGGGGCCAGGGAACCCTGGTGACAGTGTCCA GCGCTAGCGTGGCCGCTCCCAGCGTGTTCATCTTCCCACCCAGCGACG AGCAGCTGAAGTCCGGCACAGCCAGCGTGGTGTGCCTGCTGAACAACT TCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGC AGAGCGGCAACAGCCAGGAATCCGTGACCGAGCAGGACAGCAAGGAC TCCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTAC GAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTC CAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGC	
Drozitumab LC pETR7303	AGCGAGCTGACCCAGGATCCTGCCGTGTCTGTGGCTCTGGGCCAGACC GTGCGGATCACCTGTAGCGGCGACAGCCTGCGGAGCTACTACGCCAGC TGGTATCAGCAGAAGCCCGGCCAGGCTCCCGTGCTGGTGATCTACGGC GCCAACAACAGACCCAGCGGCATCCCCGACAGATTCAGCGGCAGCAG CAGCGGCAATACCGCCAGCCTGACCATCACAGGCGCCCAGGCCGAGG ACGAGGCCGACTACTACTGCAACAGCGCCGACAGCTCCGGCAACCAC GTGGTGTTCGGCGGAGGCACCAAGCTGACCGTCCTAGGTCAGCCCAAA GCCGCCCCTAGCGTGACCCTGTTCCCCCCAAGCAGCGAGGAACTGCAG GCCAACAAGGCCACCCTGGTGTGCCTGATCAGCGACTTCTACCCTGGC GCCGTGACAGTGGCCTGGAAGGCCGACTCTAGCCCTGTGAAGGCCGGC GTGGAGACAACCACCCCCAGCAAGCAGAGCAACAACAAGTACGCCGC CAGCAGCTACCTGAGCCTGACCCCCGAGCAGTGGAAGTCCCACCGGTC CTACAGCTGCCAGGTGACACACGAGGGCAGCACCGTGGAGAAAACCG TGGCCCCCACCGAGTGCAGC	221
FAP (4G8)	GAGATCGTGCTGACCCAGTCTCCCGGCACCCTGAGCCTGAGCCCTGGC	222

Description	Nucleotide sequences	SEQ ID NO
_VLCH1	GAGAGAGCCACCCTGAGCTGCAGAGCCAGCCAGAGCGTGAGCCGGAG CTACCTGGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCT GATCATCGGCGCCAGCACCCGGGCCACCGGCATCCCCGATAGATTCAG CGGCAGCGGCTCCGGCACCGACTTCACCCTGACCATCAGCCGGCTGGA ACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCCAGGTGATCCC CCCCACCTTCGGCCAGGGCACCAAGGTGGAAATCAAGAGCTCCGCTAG CACCAAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCAC CTCTGGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCC CGAGCCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGT GCACACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAG CAGCGTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACAT CTGCAACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGG TGGAACCCAAGAGCTGCGAC	
Drozitumab-FAP (4G8) VLCH1 2+2	GAAGTGCAGCTGGTGCAGTCTGGCGGCGGAGTGGAAAGACCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGTCTTGGGTCCGCCAGGCCCCTGGAAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGCGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGAGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACTGTGTCTAGCGCTAGCACCAAGGGCCCA AGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGAAC AGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTGAC AGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTTCC AGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTCAC AGTGCCTAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGA GCTGCGACAAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCT GGGCGGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCT GATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTC CCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGA GGTGCACAATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCA CCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCC CCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCC CAGGTGTACACCCTGCCCCCCCAGCAGAGATGAGCTGACCAAGAACCA GGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCAGCGATATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCAC CCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACTCCAAACTG ACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAG CGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAG CCTGAGCCCCGGCAAGTCCGGAGGCGGCGGAAGCGGAGGAGGAGGAT CCGGAGGAGGGGGAAGTGGCGGCGGAGGATCTGAGATCGTGCTGACC CAGTCTCCCGGCACCCTGAGCCTGAGCCCTGGCGAGAGAGCCACCCTG AGCTGCAGAGCCAGCCAGAGCGTGAGCCGGAGCTACCTGGCCTGGTAT CAGCAGAAGCCCGGCCAGGCCCCCAGACTGCTGATCATCGGCGCCAGC ACCCGGGCCACCGGCATCCCCGATAGATTCAGCGGCAGCGGCTCCGGC ACCGACTTCACCCTGACCATCAGCCGGCTGGAACCCGAGGACTTCGCC GTGTACTACTGCCAGCAGGGCCAGGTGATCCCCCCCACCTTCGGCCAG GGCACCAAGGTGGAAATCAAGGCTAGCACCAAGGGCCCCTCCGTGTTT CCTCTGGCCCCCAGCAGCAAGAGCACCTCTGGCGGAACAGCCGCCCTG	223

Description	Nucleotide sequences	SEQ ID NO
	GGCTGCCTGGTGAAAGACTACTTCCCCGAGCCCGTGACCGTGTCCTGG AACTCTGGCGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTG CAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGACAGTGCCCTCC AGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCC AGCAACACCAAAGTGGACAAGAAGGTGGAACCCAAGAGCTGCGAC	
FAP (4G8) _VHCL	GAGGTGCAGCTGCTGGAATCTGGAGGCGGCCTGGTGCAGCCTGGCGGC AGCCTGAGACTGTCTTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTAC GCCATGAGCTGGGTCCGACAGGCTCCTGGCAAGGGACTGGAATGGGTG TCCGCCATCTCCGGCAGCGGAGGCAGCACCTACTACGCCGACAGCGTG AAGGGCCGGTTCACCATCAGCAGAGACAACAGCAAGAACACCCTGTA CCTGCAGATGAACAGCCTGCGGGCCGAGGATACCGCCGTGTATTATTG CGCCAAGGGATGGCTGGGCAACTTCGACTACTGGGGCCAGGGAACCCT GGTGACAGTGTCCAGCGCTAGCGTGGCCGCTCCCAGCGTGTTCATCTTC CCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGTGGTGTG CCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGT GGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGACCGAGC AGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGACCCTGA GCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACC CACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAG TGC	224
Drozitumab -FAP (28H1) VHCL pETR9551 2+2	GAAGTGCAGCTGGTGCAGTCTGGCGGCGGAGTGGAAAGACCTGGCGG CAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTTCACCTTCGACGACTA CGCCATGTCTTGGGTCCGCCAGGCCCCTGGAAAGGGCCTGGAATGGGT GTCCGGCATCAACTGGCAGGGCGGCAGCACCGGCTACGCCGACAGCG TGAAGGGCAGAGTGACCATCAGCCGGGACAACGCCAAGAACAGCCTG TACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTAC TGCGCCAAGATCCTGGGAGCCGGCAGAGGCTGGTACTTCGACTACTGG GGCAAGGGCACCACCGTGACTGTGTCTAGCGCTAGCACCAAGGGCCCA AGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGAAC AGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTGAC AGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTTCC AGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTCAC AGTGCCTAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAA CCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGA GCTGCGACAAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCT GGGCGGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCT GATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTC CCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGA GGTGCACAATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCA CCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGA ACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCC CCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCC CAGGTGTACACCCTGCCCCCCAGCAGAGATGAGCTGACCAAGAACCA GGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCAGCGATATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCAC CCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACTCCAAACTG ACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAG CGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAG CCTGAGCCCCGGCAAGTCCGGAGGCGGCGGAAGCGGAGGAGGAGGAT CCGGAGGAGGGGGAAGTGGCGGCGGAGGATCTGAGGTGCAGCTGCTG GAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGATCTCTGAGACTGTCC	225

Description	Nucleotide sequences	SEQ ID NO
	TGCGCCGCCTCCGGCTTCACCTTCTCCTCCCACGCCATGTCCTGGGTCC GACAGGCTCCTGGCAAAGGCCTGGAATGGGTGTCCGCCATCTGGGCCT CCGGCGAGCAGTACTACGCCGACTCTGTGAAGGGCCGGTTCACCATCT CCCGGGACAACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGC GGGCCGAGGACACCGCCGTGTACTACTGTGCCAAGGGCTGGCTGGGCA ACTTCGACTACTGGGGCCAGGGCACCCTGGTCACCGTGTCCAGCGCTA GCGTGGCCGCTCCCAGCGTGTTCATCTTCCCACCCAGCGACGAGCAGC TGAAGTCCGGCACAGCCAGCGTGGTGTGCCTGCTGAACAACTTCTACC CCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAATCCGTGACCGAGCAGGACAGCAAGGACTCCAC CTACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGACTACGAGA AGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCCAGCC CCGTGACCAAGAGCTTCAACCGGGGCGAGTGC	
FAP (28H1) _VLCH1 pETR9537	GAGATCGTGCTGACCCAGTCTCCCGGCACCCTGAGCCTGAGCCCTGGC GAGAGAGCCACCCTGAGCTGCAGAGCCAGCCAGAGCGTGAGCCGGAG CTACCTGGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCT GATCATCGGCGCCAGCACCCGGGCCACCGGCATCCCCGATAGATTCAG CGGCAGCGGCTCCGGCACCGACTTCACCCTGACCATCAGCCGGCTGGA ACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCCAGGTGATCCC CCCCACCTTCGGCCAGGGCACCAAGGTGGAAATCAAGAGCTCCGCTAG CACCAAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCAC CTCTGGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCC CGAGCCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGT GCACACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAG CAGCGTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACAT CTGCAACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGG TGGAACCCAAGAGCTGCGAC	226
DR5 (22E9)-FAP (28H1) VHCL pETR9711 2+2	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGTGTGCGGATTTCGTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC TGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA	227

Description	Nucleotide sequences	SEQ ID NO
	ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGT CCGGAGGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAAG TGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGCCT GGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTT CACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCAAA GGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACTAC GCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAG AACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCC GTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGC CAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGC GTGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTG CAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATC CGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCT GCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCA ACCGGGGCGAGTGC	
DR5 (22E9) LC pETR9076	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTTCTAATCAGCCCGT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGG AGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGT	228
DR5 (21H3)-FAP (28H1) VHCL pETR10626 2+2	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACATGGCCGTATATTACTGTG CGAAAGGTGCTCGTGTTTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA	229

Description	Nucleotide sequences	SEQ ID NO
	GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC TGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGT CCGGAGGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAAG TGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGCCT GGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTT CACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCAAA GGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACTAC GCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAG AACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCC GTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGC CAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGC GTGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTG CAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATC CGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCT GCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCA ACCGGGGCGAGTGC	
DR5 (21H3) LC pETR9075	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCGGTGTATTACTGTCAGCAGGGTTCTCAGCCGCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGG AGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGT	230
DR5 (20F2)-FAP (28H1) VHCL pETR10135 2+2	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGTGTGAGGAAGGGGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTG GCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAG	231

Description	Nucleotide sequences	SEQ ID NO
	CGGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAG CCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAA CACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCC ACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGT GTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGAC CCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGA AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAA GACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGT CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTG CCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGT CTGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGC AACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGC CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG TCCGGAGGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAA GTGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGC CTGGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGC TTCACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCA AAGGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACT ACGCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCA AGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCG CCGTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGG GCCAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCA GCGTGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAG CCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGG TGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAA TCCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAG CACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACG CCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCT TCAACCGGGGCGAGTGC	
DR5 (20F2) LC pETR9061	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTGAGTCGCCTCCCCC GACGTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGTGGCTG CACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGG AACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCC AAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAG GAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAG CAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCT ACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGA GCTTCAACAGGGGAGAGTGT	232
DR5(5E11) -FAP (28H1)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT	233

Description	Nucleotide sequences	SEQ ID NO
VHCL pETR1033 4 2+2	CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC TGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAGT CCGGAGGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAAG TGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGCCT GGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTT CACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCAAA GGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACTAC GCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAG AACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCC GTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGC CAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGC GTGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTG CAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATC CGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCA CCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCT GCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCA ACCGGGGCGAGTGC	
DR5(5E11) LC pETR9044	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTACTACTCATCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGG	234

Description	Nucleotide sequences	SEQ ID NO
	AGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGT	
DR5(5E11)-FAP (28H1) VHCL 2+2 Removal of C-term. Lysine in Fc pETR11052	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC TCGTGAAAGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACT CCGACGGCTCATTCTTCCTGTACTCTAAGCTGACAGTGGACAAGTCCCG GTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCT GCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCCGGGGGAGG CGGAGGATCTGGCGGAGGCGGATCCGGTGGTGGCGGATCTGGGGGCG GTGGATCTGAGGTGCAGCTGCTGGAATCTGGGGGAGGACTGGTGCAGC CAGGCGGATCTCTGAGGCTGTCCTGCGCTGCTTCCGGCTTTACCTTCTC CAGCCACGCCATGAGTTGGGTGCGCCAGGCACCCGGAAAAGGACTGG AATGGGTGTCAGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGATA GCGTGAAGGGCCGGTTCACCATCTCTCGGGATAACAGCAAGAATACTC TGTACCTGCAGATGAACTCCCTGCGCGCTGAAGATACCGCTGTGTATT ACTGCGCCAAGGGCTGGCTGGGCAACTTCGATTACTGGGGCCAGGGAA CCCTCGTGACTGTCTCGAGCGCTTCTGTGGCCGCTCCCTCCGTGTTCAT CTTCCCACCTTCCGACGAGCAGCTGAAGTCCGGCACTGCCTCTGTCGTG TGCCTGCTGAACAACTTCTACCCTCGGGAAGCCAAGGTGCAGTGGAAA GTGGATAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTGACCGAG CAGGACTCCAAGGACAGCACCTACTCCCTGAGCAGCACCCTGACCCTG TCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGTGAAGTGACC CACCAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAG TGC	235
DR5(5E11)-FAP (28H1)	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT	236

Description	Nucleotide sequences	SEQ ID NO
VHCL 2+2 Removal of C-term. Lysine in Fc P329G/LA LA mut. pETR11025	CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCATCCGTGTTCCCTCTGGC CCCTTCCAGCAAGTCTACCTCTGGCGGCACAGCCGCTCTGGGCTGCCTC GTGAAGGACTACTTCCCCGAGCCTGTGACAGTGTCCTGGAACTCTGGC GCCCTGACATCCGGCGTGCACACCTTTCCAGCTGTGCTGCAGTCCTCCG GCCTGTACTCCCTGTCCTCCGTCGTGACAGTGCCCTCCAGCTCTCTGGG CACCCAGACCTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAA GGTGGACAAGAAGGTGGAACCCAAGTCCTGCGACAAGACCCACACCT GTCCCCCTTGTCCTGCCCCTGAAGCTGCTGGCGGCCCTAGCGTGTTCCT GTTCCCCCCAAAGCCCAAGGACACCCTGATGATCTCCCGGACCCCCGA AGTGACCTGCGTGGTGGTGGATGTGTCCCACGAGGACCCTGAAGTGAA GTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAATGCCAAGACCAA GCCTAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCT GACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCA AGGTGTCCAACAAGGCCCTGGGAGCCCCCATCGAAAAGACCATCTCCA AGGCCAAGGGCCAGCCTCGCGAGCCTCAGGTGTACACCCTGCCCCCTA GCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGA AAGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGAGCAACGGCC AGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACG GCTCATTCTTCCTGTACTCTAAGCTGACAGTGGACAAGTCCCGGTGGCA GCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAA CCACTACACCCAGAAGTCCCTGTCCCTGTCTCCCGGGGGGAGGCGGAGG ATCTGGCGGAGGCGGATCCGGTGGTGGCGGATCTGGGGGCGGTGGATC TGAGGTGCAGCTGCTGGAATCTGGGGGAGGACTGGTGCAGCCAGGCG GATCTCTGAGGCTGTCCTGCGCTGCTTCCGGCTTTACCTTCTCCAGCCA CGCCATGAGTTGGGTGCGCCAGGCACCCGGAAAAGGACTGGAATGGG TGTCAGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGATAGCGTGA AGGGCCGGTTCACCATCTCTCGGGATAACAGCAAGAATACTCTGTACC TGCAGATGAACTCCCTGCGCGCTGAAGATACCGCTGTGTATTACTGCG CCAAGGGCTGGCTGGGCAACTTCGATTACTGGGGCCAGGGAACCCTCG TGACTGTCTCGAGCGCTTCTGTGGCCGCTCCCTCCGTGTTCATCTTCCC ACCTTCCGACGAGCAGCTGAAGTCCGGCACTGCCTCTGTCGTGTGCCT GCTGAACAACTTCTACCCTCGGGAAGCCAAGGTGCAGTGGAAAGTGGA TAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTGACCGAGCAGGA CTCCAAGGACAGCACCTACTCCCTGAGCAGCACCCTGACCCTGTCCAA GGCCGACTACGAGAAGCACAAGGTGTACGCCTGTGAAGTGACCCACC AGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGC	
VHVL Cross DR5(5E11)-FAP (28H1) pETR11827 2+2	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTACTACTCATCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAAAGCTCCGCTAGCAC CAAGGGCCCAAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAG CGGCGGAACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGA GCCCGTGACAGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCA CACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAG	237

Description	Nucleotide sequences	SEQ ID NO
	CGTGGTCACAGTGCCTAGCAGCAGCCTGGGCACCCAGACCTACATCTG CAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGG AGCCCAAGAGCTGCGACAAGACCCACACCTGTCCCCCTTGTCCTGCCC CTGAGCTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCA AGGACACCCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGG TGGACGTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGG ACGGCGTGGAGGTGCACAATGCCAAGACCAAGCCCCGGGAGGAACAG TACAACAGCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAG GACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGC CCTGCCTGCCCCCATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCC CAGAGAACCCCAGGTGTACACCCTGCCCCCCAGCAGAGATGAGCTGAC CAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCAG CGATATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCTGAGAACAACT ACAAGACCACCCCCCCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGT ACTCCAAACTGACCGTGGACAAGAGCCGGTGGCAGCAGGGCAACGTG TTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAG AAGTCCCTGAGCCTGAGCCCCGGCAAGTCCGGAGGCGGCGGAAGCGG AGGAGGAGGATCCGGAGGAGGGGGAAGTGGCGGCGGAGGATCTGAG GTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGGATCT CTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCTCCTCCCACGCCA TGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCTGGAATGGGTGTCCG CCATCTGGGCCTCCGGCGAGCAGTACTACGCCGACTCTGTGAAGGGCC GGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTACCTGCAGA TGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCCAAGG GCTGGCTGGGCAACTTCGACTACTGGGGCCAGGGCACCCTGGTCACCG TGTCCAGCGCTAGCACCAAGGGCCCCTCCGTGTTTCCTCTGGCCCCTAG CTCTAAGAGCACCAGCGGAGGAACAGCCGCCCTGGGCTGCCTCGTGAA AGACTACTTCCCCGAGCCCGTGACAGTGTCTTGGAACTCTGGCGCCCT GACCAGCGGCGTGCACACATTTCCAGCTGTGCTGCAGTCCAGCGGCCT GTACTCTCTGAGCAGCGTCGTGACTGTGCCCAGCTCTAGCCTGGGAAC CCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAACACCAAAGT GGATAAGAAGGTGGAACCCAAGAGCTGCGAC	
DR5(5E11) VHCL pETR11484	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCGTGGCTGCACCATCTGTCTTCATCTTCCC GCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTG CTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGAT AACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGA CAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCA AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT CAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT	238
FAP (28H1) VLCL pETR9366	GAGATCGTGCTGACCCAGTCCCCCGGCACCCTGTCTCTGAGCCCTGGC GAGAGAGCCACCCTGTCCTGCAGAGCCTCCCAGTCCGTGTCCCGGTCC TACCTCGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCTCGGCTGCTG ATCATCGGCGCCTCTACCAGAGCCACCGGCATCCCTGACCGGTTCTCC GGCTCTGGCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTGGAA	239

Description	Nucleotide sequences	SEQ ID NO
	CCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCCAGGTCATCCCT CCCACCTTTGGCCAGGGCACCAAGGTGGAAATCAAGCGTACGGTGGCT GCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG GAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGC CAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCA GGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCA GCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG AGCTTCAACAGGGGAGAGTGT	
CH1CL Cross DR5(5E11) -FAP (28H1) pETR11828 2+2	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCGTGGCCGCTCCCAGCGTGTTCATCTTCCC ACCTAGCGACGAGCAGCTGAAGTCCGGCACAGCCTCTGTCGTGTGCCT GCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGA CAATGCCCTGCAGAGCGGCAACAGCCAGGAAAGCGTGACCGAGCAGG ACAGCAAGGACTCCACCTACAGCCTGAGCAGCACACTGACCCTGAGCA AGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCAC CAGGGCCTGTCTAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGC GACAAGACCCACACCTGCCCCCCTTGTCCTGCCCCTGAACTGCTGGGA GGCCCTAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATG ATCAGCCGGACCCCTGAAGTGACCTGCGTGGTGGTGGATGTGTCCCAC GAGGACCCAGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGT GCACAATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCT ACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACG GCAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCA TCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAG GTGTACACCCTGCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTG TCCCTGACCTGTCTCGTGAAAGGCTTCTACCCCTCCGATATCGCCGTGG AATGGGAGAGCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCC CCTGTGCTGGACTCCGACGGCTCATTCTTCCTGTACTCTAAGCTGACAG TGGACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGA TGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGT CTCCCGGGGGAGGCGGAGGATCTGGCGGAGGCGGATCCGGAGGAGGG GGAAGTGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGG AGGCCTGGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTC CGGCTTCACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCT GGCAAAGGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAG TACTACGCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAAC TCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGAC ACCGCCGTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTAC TGGGGCCAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCACCAAGGGC CCCTCCGTGTTTCCTCTGGCCCCTAGCTCTAAGAGCACCAGCGGAGGA ACAGCCGCCCTGGGCTGCCTCGTGAAAGACTACTTCCCCGAGCCCGTG ACAGTGTCTTGGAACTCTGGCGCCCTGACCAGCGGCGTGCACACATTT CCAGCTGTGCTGCAGTCCAGCGGCCTGTACTCTCTGAGCAGCGTCGTG ACTGTGCCCAGCTCTAGCCTGGGAACCCAGACCTACATCTGCAACGTG	240

Description	Nucleotide sequences	SEQ ID NO
	AACCACAAGCCCAGCAACACCAAAGTGGATAAGAAGGTGGAACCCAA GAGCTGCGAC	
DR5(5E11) VLCH1 pETR11480	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTACTACTCATCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAAAGCTCCGCTAGCAC CAAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCACCTC TGGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCCCGA GCCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGTGCA CACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAG CGTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACATCTG CAACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGGTGG AACCCAAGAGCTGCGAC	241
DR5(18F11)-FAP (28H1) VHCL 2+2 pETR9801	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGTATATTACTGTG CGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTG GCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAG CGGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAG CCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAA CACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCC ACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGT GTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGAC CCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGA AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAA GACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGT CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTG CCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGT CTGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGC AACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGC CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG TCCGGAGGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAA GTGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGC CTGGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGC TTCACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCA AAGGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACT ACGCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCA AGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCG	242

Description	Nucleotide sequences	SEQ ID NO
	CCGTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGG GCCAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCA GCGTGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAG CCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGG TGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAA TCCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAG CACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACG CCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCT TCAACCGGGGCGAGTGC	
DR5(18F11) LC pETR9070	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTCAGTTGCCTCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGG AGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGT	243
DR5(5E11)-FAP (28H1) Fc knob VHCL 2+1 pETR10427	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCCTGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGTGGTGTC TGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCGGAG	244

Description	Nucleotide sequences	SEQ ID NO
	GCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGGAAGTGGCGG CGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCA GCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTC TCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCTG GAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGAC TCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACC CTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTAC TACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGCCAGGGC ACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGCGTGTTC ATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGTG GTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGAC CGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGA CCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAA GTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGG GGCGAGTGC	
DR5(5E11) Fc hole pETR1033 6	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTGCACCCTGC CCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGAGCTGTG CCGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGGTGTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG	245
DR5(5E11) -FAP (28H1) Fc knob VHCL 3+1 pETR1042	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG	246

Description	Nucleotide sequences	SEQ ID NO
7	TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCCTGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGTGGTGTC TGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCGGAG GCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAAGTGGCGG CGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCA GCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTC TCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCTG GAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGAC TCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACC CTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTAC TACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGCCAGGGC ACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGCGTGTTC ATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGTG GTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGG AAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGAC CGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGA CCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAA GTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGG GGCGAGTGC	
DR5(5E11)-DR5(5E11) Fc hole pETR10429	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA	247

Description	Nucleotide sequences	SEQ ID NO
	CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTGCACCCTGC CCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGAGCTGTG CCGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACA GCGACGGCAGCTTCTTCCTGGTGTCCAAACTGACCGTGGACAAGAGCC GGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCCC TGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCGGAG GCGGCGGAAGCGGAGGAGGAGGATCCGGAGGGGGAGGATCTGGCGG AGGCGGCAGCGAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACA GCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTT AGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTG GAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCA GACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAAC ACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTA TATTACTGTGCGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAA GGAACCCTGGTCACCGTCTCGAGTGCTAGCACCAAGGGCCCCTCCGTG TTTCCTCTGGCCCCCAGCAGCAAGAGCACCTCTGGCGGAACAGCCGCC CTGGGCTGCCTGGTGAAAGACTACTTCCCCGAGCCCGTGACCGTGTCC TGGAACTCTGGCGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTG CTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGACAGTGCCC TCCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAG CCCAGCAACACCAAAGTGGACAAGAAGGTGGAACCCAAGAGCTGCGA C	
DR5(5E11) _Fc knob Fab-Fab Head-to- tail 2+1 pETR1066 2	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGG CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAG GCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCT CAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCT TGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA CCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGGAGGCGGAGGA TCCGGCGGAGGCGGATCTGAGGTGCAATTGTTGGAGTCTGGGGGAGGC TTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGA TTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGG AAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACA TACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAAT TCCAAGAACACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGAC ACGGCCGTATATTACTGTGCGAAAGGGGTGAGGGTGTCTTTTGACTAC	248

Description	Nucleotide sequences	SEQ ID NO
	TGGGGCCAAGGAACCCTGGTCACCGTCTCGAGTGCTAGCACCAAGGGC CCAAGCGTGTTCCCTCTGGCCCCCAGCAGCAAGAGCACAAGCGGCGGA ACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAGCCCGTG ACAGTGTCCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTTT CCAGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTC ACAGTGCCTAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTG AACCACAAGCCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAA GAGCTGCGACAAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAGCT GCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACAC CCTGATGATCAGCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGT GTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGT GGAAGTGCACAATGCCAAGACCAAGCCGCGGGAGGAGCAGTACAACA GCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGC TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAG CCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATGCCGGGATGAGCTGACCAAGAAC CAGGTCAGCCTGTGGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC GCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAG CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC TCCCTGTCTCCGGGTAAA	
FAP (28H1) _Fc hole VHCL Cross pETR10130	GAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGG ATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCTCCTCCCAC GCCATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCTGGAATGGGTG TCCGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGACTCTGTGAAG GGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTACCTG CAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCC AAGGGCTGGCTGGGCAACTTCGACTACTGGGGACAGGGCACCCTGGTC ACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGCGTGTTCATCTTCCCAC CCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGTGGTGTGCCTGC TGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACA ACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGACCGAGCAGGAC AGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAG GCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCA GGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGCGA CAAGACCCACACCTGTCCCCCTTGCCCTGCCCCTGAACTGCTGGGTGG CCCTTCCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATC AGCCGGACCCCCGAAGTGACCTGCGTGGTGGTCGATGTGTCCCACGAG GACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC AATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCG GGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAA AGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGA GAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGT GCACCCTGCCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCC TGAGCTGTGCCGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGT GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCT GTGCTGGACAGCGACGGCAGCTTCTTCCTGGTGTCCAAACTGACCGTG GACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGAT GCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAG CCCCGGCAAG	249

Description	Nucleotide sequences	SEQ ID NO
DR5(18F11)-FAP (28H1) Fc knob VHCL 2+1 pETR9807	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGTATATTACTGTG CGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTG GCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAG CGGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAG CCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAA CACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCC ACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGT GTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGAC CCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGA AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAA GACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGT CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTG CCCCCCTGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGTGGTGT CTGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGC AACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGC CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG TCCGGAGGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAA GTGGCGGCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGC CTGGTGCAGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGC TTCACCTTCTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCA AAGGCCTGGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACT ACGCCGACTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCA AGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCG CCGTGTACTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGG GCCAGGGCACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCA GCGTGTTCATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAG CCAGCGTGGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGG TGCAGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAA TCCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAG CACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACG CCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCT TCAACCGGGGCGAGTGC	250
DR5(18F11) Fc hole pETR9808	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGTATATTACTGTG CGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCAAGGAACCCTG	251

Description	Nucleotide sequences	SEQ ID NO
	GTCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTG GCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAG CGGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAG CCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAA CACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCC ACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGT GTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGAC CCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGA AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAA GACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGT CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTGCACCCTG CCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGAGCTGT GCCGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGC AACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGAC AGCGACGGCAGCTTCTTCCTGGTGTCCAAACTGACCGTGGACAAGAGC CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCAAG	
DR5(18F11)-FAP (28H1) VHCL Fc knob 3+1 pETR10333	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGTATATTACTGTG CGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTG GCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAG CGGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAG CCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAA CACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCC ACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGT GTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGAC CCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGA AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAA GACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGT CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTG CCCCCCTGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGTGGTGT CTGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGC AACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGAC AGCGACGGCAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGAGC CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCGGA GGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGAGGGGGAAGTGGCG GCGGAGGATCTGAGGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGC	252

Description	Nucleotide sequences	SEQ ID NO
	AGCCTGGCGGATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTT CTCCTCCCACGCCATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCT GGAATGGGTGTCCGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGA CTCTGTGAAGGGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACAC CCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTA CTACTGTGCCAAGGGCTGGCTGGGCAACTTCGACTACTGGGGCCAGGG CACCCTGGTCACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGCGTGTT CATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGT GGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTG GAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGA CCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTG ACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA AGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCG GGGCGAGTGC	
DR5(18F11)-DR5(18F11) Fc hole pETR10288	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGTATATTACTGTG CGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCAAGGAACCCTG GTCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTG GCCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTG CCTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAG CGGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAG CCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAA CACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCC ACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGT GTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGAC CCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGA AGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAA GACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGT CCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACA AGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCA TCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTGCACCCTG CCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGAGCTGT GCCGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTGGGAGAGC AACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCTGTGCTGGAC AGCGACGGCAGCTTCTTCCTGGTGTCCAAACTGACCGTGGACAAGAGC CGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGATGCACGAGGCC CTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAGCCCCGGCGGA GGCGGCGGAAGCGGAGGAGGAGGATCCGGAGGGGGAGGATCTGGCG GAGGCGGCAGCGAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTAC AGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTT TAGCAGTTATGCCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCT GGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGC AGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAA CACGCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGACGCGGCCGT ATATTACTGTGCGAAAGGGGTGCGTAAGAAGTTTGACTACTGGGGCCA AGGAACCCTGGTCACCGTCTCGAGTGCTAGCACCAAGGGCCCCTCCGT	253

Description	Nucleotide sequences	SEQ ID NO
	GTTTCCTCTGGCCCCCAGCAGCAAGAGCACCTCTGGCGGAACAGCCGC CCTGGGCTGCCTGGTGAAAGACTACTTCCCCGAGCCCGTGACCGTGTC CTGGAACTCTGGCGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGT GCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGCGTGGTGACAGTGCC CTCCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAA GCCCAGCAACACCAAAGTGGACAAGAAGGTGGAACCCAAGAGCTGCG AC	
hu Fc _wt	GCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAG AGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTAC TTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAG AAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGC CCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAA AACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCG TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT ACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAG GAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG CACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGG GCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGA GCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTA TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGA ACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT TCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACA CGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA	254
hu Fc_P329G/ LALA	GCTAGCACCAAGGGCCCATCCGTGTTCCCTCTGGCCCCTTCCAGCAAG TCTACCTCTGGCGGCACAGCCGCTCTGGGCTGCCTCGTGAAGGACTAC TTCCCCGAGCCTGTGACAGTGTCCTGGAACTCTGGCGCCCTGACATCC GGCGTGCACACCTTTCCAGCTGTGCTGCAGTCCTCCGGCCTGTACTCCC TGTCCTCCGTCGTGACAGTGCCCTCCAGCTCTCTGGGCACCCAGACCTA CATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGA AGGTGGAACCCAAGTCCTGCGACAAGACCCACACCTGTCCCCCTTGTC CTGCCCCTGAAGCTGCTGGCGGCCCTAGCGTGTTCCTGTTCCCCCCAAA GCCCAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGT GGTGGTGGATGTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTA CGTGGACGGCGTGGAAGTGCACAATGCCAAGACCAAGCCTAGAGAGG AACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGC ACCAGGATTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAAC AAGGCCCTGGGAGCCCCCATCGAAAAGACCATCTCCAAGGCCAAGGG CCAGCCTCGCGAGCCTCAGGTGTACACCCTGCCCCCTAGCAGAGATGA GCTGACCAAGAACCAGGTGTCCCTGACCTGTCTCGTGAAAGGCTTCTA CCCCTCCGATATCGCCGTGGAATGGGAGAGCAACGGCCAGCCCGAGA ACAACTACAAGACCACCCCCCCTGTGCTGGACTCCGACGGCTCATTCT TCCTGTACTCTAAGCTGACAGTGGACAAGTCCCGGTGGCAGCAGGGCA ACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACA CCCAGAAGTCCCTGTCCCTGTCTCCCGGGAAA	255
hu kappa light chain	CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTA	256

Description	Nucleotide sequences	SEQ ID NO
	TCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATC GGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCG CCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT	
hu lambda light chain	GGTCAGCCCAAAGCCGCCCCTAGCGTGACCCTGTTCCCCCCAAGCAGC GAGGAACTGCAGGCCAACAAGGCCACCCTGGTGTGCCTGATCAGCGA CTTCTACCCTGGCGCCGTGACAGTGGCCTGGAAGGCCGACTCTAGCCC TGTGAAGGCCGGCGTGGAGACAACCACCCCCAGCAAGCAGAGCAACA ACAAGTACGCCGCCAGCAGCTACCTGAGCCTGACCCCCGAGCAGTGGA AGTCCCACCGGTCCTACAGCTGCCAGGTGACACACGAGGGCAGCACCG TGGAGAAAACCGTGGCCCCCACCGAGTGCAGC	257
28H1_Fc hole VHCL pETR10130	GAAGTGCAGCTGCTGGAATCCGGCGGAGGCCTGGTGCAGCCTGGCGG ATCTCTGAGACTGTCCTGCGCCGCCTCCGGCTTCACCTTCTCCTCCCAC GCCATGTCCTGGGTCCGACAGGCTCCTGGCAAAGGCCTGGAATGGGTG TCCGCCATCTGGGCCTCCGGCGAGCAGTACTACGCCGACTCTGTGAAG GGCCGGTTCACCATCTCCCGGGACAACTCCAAGAACACCCTGTACCTG CAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCC AAGGGCTGGCTGGGCAACTTCGACTACTGGGGACAGGGCACCCTGGTC ACCGTGTCCAGCGCTAGCGTGGCCGCTCCCAGCGTGTTCATCTTCCCAC CCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGTGGTGTGCCTGC TGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACA ACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGACCGAGCAGGAC AGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTGACCCTGAGCAAG GCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCA GGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGCGA CAAGACCCACACCTGTCCCCCTTGCCCTGCCCCTGAACTGCTGGGTGG CCCTTCCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATC AGCCGGACCCCCGAAGTGACCTGCGTGGTGGTCGATGTGTCCCACGAG GACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCAC AATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCG GGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAA AGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGA GAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGT GCACCCTGCCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCC TGAGCTGTGCCGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGT GGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCCCCT GTGCTGGACAGCGACGGCAGCTTCTTCCTGGTGTCCAAACTGACCGTG GACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGTGAT GCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCTGAG CCCCGGCAAG	258
28H1 VLCH1 pETR9537	GAGATCGTGCTGACCCAGTCTCCCGGCACCCTGAGCCTGAGCCCTGGC GAGAGAGCCACCCTGAGCTGCAGAGCCAGCCAGAGCGTGAGCCGGAG CTACCTGGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCCAGACTGCT GATCATCGGCGCCAGCACCCGGGCCACCGGCATCCCCGATAGATTCAG CGGCAGCGGCTCCGGCACCGACTTCACCCTGACCATCAGCCGGCTGGA ACCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCCAGGTGATCCC CCCCACCTTCGGCCAGGGCACCAAGGTGGAAATCAAGAGCTCCGCTAG CACCAAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCAC CTCTGGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCC CGAGCCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGT	259

Description	Nucleotide sequences	SEQ ID NO
	GCACACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAG CAGCGTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACAT CTGCAACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGG TGGAACCCAAGAGCTGCGAC	
5E11_4B9 VHCL pETR1106 0	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTGCACAATGCCAAG ACCAAGCCCCGGGAGGAACAGTACAACAGCACCTACCGGGTGGTGTC CGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAA GTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCAT CAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGGTGTACACCCTGC CCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGTC TCGTGAAAGGCTTCTACCCCTCCGATATCGCCGTGGAATGGGAGAGCA ACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTGGACT CCGACGGCTCATTCTTCCTGTACTCTAAGCTGACAGTGGACAAGTCCCG GTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCT GCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCCGGGGGAGG CGGAGGATCTGGCGGAGGCGGATCCGGAGGAGGGGGAAGTGGCGGCG GAGGATCTGAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGC CTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAG CAGTTATGCTATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGA GTGGGTCTCAGCTATTATTGGTAGTGGTGCCAGCACATACTACGCAGA CTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACAC GCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATA TTACTGTGCGAAAGGGTGGTTTGGTGGTTTTAACTACTGGGGCCAAGG AACCCTGGTCACCGTCTCGAGTGCTAGCGTGGCCGCTCCCAGCGTGTT CATCTTCCCACCCAGCGACGAGCAGCTGAAGTCCGGCACAGCCAGCGT GGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTG GAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCCAGGAATCCGTGA CCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGCACCCTG ACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA AGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCG GGGCGAGTGC	260
4B9 VLCH1 pETR1002	GAGATCGTGCTGACCCAGTCCCCCGGCACCCTGTCTCTGAGCCCTGGC GAGAGAGCCACCCTGTCCTGCAGAGCCTCCCAGTCCGTGACCTCCTCC TACCTCGCCTGGTATCAGCAGAAGCCCGGCCAGGCCCCTCGGCTGCTG	261

Description	Nucleotide sequences	SEQ ID NO
0	ATCAACGTGGGCAGTCGGAGAGCCACCGGCATCCCTGACCGGTTCTCC GGCTCTGGCTCCGGCACCGACTTCACCCTGACCATCTCCCGGCTGGAA CCCGAGGACTTCGCCGTGTACTACTGCCAGCAGGGCATCATGCTGCCC CCCACCTTTGGCCAGGGCACCAAGGTGGAAATCAAGAGCTCCGCTAGC ACCAAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCACC TCTGGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCCC GAGCCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGTG CACACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGC AGCGTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACATC TGCAACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGGT GGAACCCAAGAGCTGCGAC	
5E11-Fc knob	GAGGTGCAATTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTCACCTTTAGCAGTTATG CCATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCT CAGCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGA AGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATC TGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTG CGAAAGGGGTGAGGGTGTCTTTTGACTACTGGGGCCAAGGAACCCTGG TCACCGTCTCGAGTGCTAGCACCAAGGGCCCAAGCGTGTTCCCTCTGG CCCCCAGCAGCAAGAGCACAAGCGGCGGAACAGCCGCCCTGGGCTGC CTGGTCAAGGACTACTTCCCCGAGCCCGTGACAGTGTCCTGGAACAGC GGAGCCCTGACCAGCGGCGTGCACACCTTTCCAGCCGTGCTGCAGAGC AGCGGCCTGTACAGCCTGAGCAGCGTGGTCACAGTGCCTAGCAGCAGC CTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCCAGCAAC ACCAAGGTGGACAAGAAGGTGGAGCCCAAGAGCTGCGACAAGACCCA CACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGCGGACCCAGCGTG TTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATGATCAGCCGGACC CCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCTGAA GTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAATGCCAAG ACCAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAG CGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCAT CTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC CCCCATGCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGTGGTGCC TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCA ATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACT CCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTC TGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA	264
5E11_LC pETR9044	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCT ACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCA TCTATGGAGCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTG GCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC CTGAAGATTTTGCAGTGTATTACTGTCAGCAGGGTACTACTCATCCCAT TACGTTCGGCCAGGGGACCAAAGTGGAAATCAAACGTACGGTGGCTGC ACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA AAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGG AGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGC AGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA	265

Description	Nucleotide sequences	SEQ ID NO
	CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAG CTTCAACAGGGGAGAGTGT	
huFc-hVH007	GACAAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGC GGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATG ATCAGCCGGACCCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCAC GAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTG CACAATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTA CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGG CAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGG TGTACACCCTGCCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGT CCCTGACCTGTCTGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGG AGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCC CCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACTCCAAACTGACC GTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGT GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCT GAGCCCCGGCGGAGGAGGCGGAAGTGGAGGCGGAGGATCCGGAGGG GGAGGATCTGGCGGAGGCGGCAGCGAAGTGCAGCTTGTCGAAACCGG CGGGGGACTCATCCAGCCCGGCGGTAGCCTGAGGCTTTCCTGCGCCGC TTCTGGGTTCACAGTGTCAAGATACGCCATGATTTGGGTCCGCCAGGC CCCTGGCAAGGGACTGGAGTATATCGGTTTTATTACCAGCGACGGCTC CACTTACTATGCTGATTCTGCAAAAGGGCGGTTCACAATCAGTAGGGA CAACAGCAAGAATACCCTCTACCTCCAGATGAACTCCTTGAGAGCCGA GGATACTGCTGTGTACTATTGTGCACGCTACACCTATTCTGACGGAACA GACCTGTGGGGCCGGGGAACCCTCGTCACTGTCTCCTCAGCTAGCACC AAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCACCTCT GGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCCCGAG CCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGTGCAC ACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGC GTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACATCTGC AACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGGTGGA ACCCAAGAGCTGCGAC	312
hVL015	GACATCCAGATGACCCAGAGCCCCTCCACACTGTCTGCTTCAGTGGGC GATAGGGTCACCATTACTTGCAGAGCAAGCCAGTCCATCTCTACATAC CTCAGTTGGTATCAGCAAAAGCCTGGGAAAGCCCCAAAGCGCCTGATT TACAAGGCCAGCTCCCTTGCATCTGGAGTGCCCTCACGGTTCAGCGGC TCCGGTTCTGGGACCGAGTTTACTCTGACCATCAGTAGCCTCCAGCCTG ACGATGCCGCTACATATTACTGTCAGCCAAACTCCGGCATAGCAACCT ACGGAGCCGCTTTCGGTGGCGGGACAAAAGTCGAAATCAAACGTACG GTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGA AATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAG AGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTAC AGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACA CAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT CACAAAGAGCTTCAACAGGGGAGAGTGT	313
huFc-hVH017	GACAAGACCCACACCTGTCCCCCTTGTCCTGCCCCTGAGCTGCTGGGC GGACCCAGCGTGTTCCTGTTCCCCCCAAAGCCCAAGGACACCCTGATG ATCAGCCGGACCCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCCAC GAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAGGTG CACAATGCCAAGACCAAGCCCCGGGAGGAACAGTACAACAGCACCTA	314

Description	Nucleotide sequences	SEQ ID NO
	CCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGG CAAAGAGTACAAGTGCAAGGTCTCCAACAAGGCCCTGCCTGCCCCCAT CGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCCAGAGAACCCCAGG TGTACACCCTGCCCCCCAGCAGAGATGAGCTGACCAAGAACCAGGTGT CCCTGACCTGTCTGGTCAAGGGCTTCTACCCCAGCGATATCGCCGTGG AGTGGGAGAGCAACGGCCAGCCTGAGAACAACTACAAGACCACCCCC CCTGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACTCCAAACTGACC GTGGACAAGAGCCGGTGGCAGCAGGGCAACGTGTTCAGCTGCAGCGT GATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGAGCCT GAGCCCCGGCGGAGGAGGCGGAAGTGGAGGCGGAGGATCCGGAGGG GGAGGATCTGGCGGAGGCGGCAGCCAAGTGCAGCTCGTCGAGAGCGG CGGGGGACTCGTGCAGCCCGGCGGTTCCCTGAGGCTTTCTTGCTCAGC CAGCGGGTTCTCCATCTCCAGATACGCTATGATTTGGGTCCGCCAGGC ACCTGGCAAGGGACTGGAATATGTGGGTTTTATCACCAGTGACAGCTC CGCCTACTATGCTTCTTGGGCAAAAGGCCGGTTCACAATTTCAAGGGA TAACAGCAAGAATACCCTCTACCTTCAAATGAACTCCTTGAGAGCCGA GGACACTGCTGTTTACTATTGTGCACGCTACACATATTCTGATGGGACC GACCTGTGGGGACAGGGCACTCTCGTCACTGTCTCCTCAGCTAGCACC AAGGGCCCCTCCGTGTTTCCTCTGGCCCCCAGCAGCAAGAGCACCTCT GGCGGAACAGCCGCCCTGGGCTGCCTGGTGAAAGACTACTTCCCCGAG CCCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGCGTGCAC ACCTTTCCAGCCGTGCTGCAGAGCAGCGGCCTGTACTCCCTGAGCAGC GTGGTGACAGTGCCCTCCAGCAGCCTGGGCACCCAGACCTACATCTGC AACGTGAACCACAAGCCCAGCAACACCAAAGTGGACAAGAAGGTGGA ACCCAAGAGCTGCGAC	
hVL011	GACATCCAGATGACCCAGAGCCCCTCCTCTCTGTCAGCCAGCGTGGGC GATAGGGTCACAATTACCTGTCAGGCTTCCCAATCTATCAGTACTTACC TGAGCTGGTATCAACAGAAGCCTGGGCAGCCACCCAAAAGACTGATTT ACAAGGCATCCACACTTGCCTCTGGAGTGCCTTCACGCTTCAGCGGCT CCGGTTCTGGGACCGACTTTACTCTGACCATCAGTAGCCTCCAGCCAG AGGATTTCGCTACATATTACTGTCAACCCAACTCCGGCATAGCAACCT ACGGAGCCGCTTTTGGTGGCGGGACAAAGGTCGAAATCAAACGTACG GTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGA AATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAG AGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTA ACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTAC AGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACA CAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT CACAAAGAGCTTCAACAGGGGAGAGTGT	315

14. DR5 Sequences

Name	Sequence	Seq ID No
Human DR5 swissprot O14763	MEQRGQNAPA ASGARKRHGP GPREARGARP GPRVPKTLVL VVAAVLLLVS AESALITQQDLAPQQRAAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG DYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP EMCRKCRTGC PRGMVKVGDC TPWSDIECVH KESGTKHSGE APAVEETVTS SPGTPASPCS LSGIIIGVTV AAVVLIVAVF VCKSLLWKKV LPYLKGICSG GGGDPERVDR SSQRPGAEDN VLNEIVSILQ PTQVPEQEME VQEPAEPTGV NMLSPGESEH LLEPAEAERS QRRRLLVPAN EGDPTETLRQ CFDDFADLVP FDSWEPLMRK LGLMDNEIKV AKAEAAGHRD TLYTMLIKWV NKTGRDASVH TLLDALETLG ERLAKQKIED HLLSSGKFMY LEGNADSAMS	155
Hu DR5 (ECD)-AcTev-Fc-Avi	ITQQDLAPQQRAAPQQKRSSPSEGLCPPGHHISEDGRDCISCKYG QDYSTHWNDLLFCLRCTRCDSGEVELSPCTTTRNTVCQCEEGTF REEDSPEMCRKCRTGCPRGMVKVGDCTPWSDIECVHKESGTKH SGEAPAVEETVTSSPGTPASVDEQLYFQGGSPKSADKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGSG GLNDIFEAQKIEWHE	316
Cynom. DR5 (ECD)-AcTev-Fc-Avi	ITRQSLDPQRRAAPQQKRSSPTEGLCPPGHHISEDSRECISCKYGQ DYSTHWNDFLFCLRCTKCDSGEVEVNSCTTTRNTVCQCEEGTFR EEDSPEICRKCRTGCPRGMVKVKDCTPWSDIECVHKESVDEQLY FQGGSPKSADKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGGGSGGLNDIFEAQKIEWHE	317

15. FAP Sequences

Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
Human FAP ectodomain+poly-lys-tag+his_6-tag	RPSRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQEYLHQSA DNNIVLYNIETGQSYTILSNRTMKSVNASNYGLSPDRQFVYLESDY SKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQYLCWSPVGSKLA YVYQNNIYLKQRPGDPPFQITFNGRENKIFNGIPDWVYEEEMLATK YALWWSPNGKFLAYAEFNDTDIPVIAYSYYGDEQYPRTINIPYPKA GAKNPVVRIFIIDTTYPAYVGPQEVPVPAMIASSDYYFSWLTWVTD ERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHIEESRTG WAGGFFVSTPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITS GKWEAINIFRVTQDSLFYSSNEFEEYPGRRNIYRISIGSYPPSKKCVT CHLRKERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIK ILEENKELENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSKK YPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAF	156

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Construct	POLYPEPTIDE SEQUENCE	SEQ ID NO
	QGDKLLYAVYRKLGVYEVEDQITAVRKFIEMGFIDEKRIAIWGWS YGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASVYTERFMGLPT KDDNLEHYKNSTVMARAEYFRNVDYLLIHGTADDNVHFQNSAQI AKALVNAQVDFQAMWYSDQNHGLSGLSTNHLYTHMTHFLKQCF SLSDGKKKKKKGHHHHHH	
Murine FAP ectodomain+poly-lys-tag+his_6-tag	RPSRVYKPEGNTKRALTLKDILNGTFSYKTYFPNWISEQEYLHQSE DDNIVFYNIETRESYIILSNSTMKSVNATDYGLSPDRQFVYLESDYS KLWRYSYTATYYIYDLQNGEFVRGYELPRPIQYLCWSPVGSKLAY VYQNNIYLKQRPGDPPFQITYTGRENRIFNGIPDWVYEEEMLATKY ALWWSPDGKFLAYVEFNDSDIPIIAYSYYGDGQYPRTINIPYPKAG AKNPVVRVFIVDTTYPHHVGPMEVPVPEMIASSDYYFSWLTWVSS ERVCLQWLKRVQNVSVLSICDFREDWHAWECPKNQEHVEESRTG WAGGFFVSTPAFSQDATSYYKIFSDKDGYKHIHYIKDTVENAIQITS GKWEAIYIFRVTQDSLFYSSNEFEGYPGRRNIYRISIGNSPPSKKCVT CHLRKERCQYYTASFSYKAKYYALVCYGPGLPISTLHDGRTDQEIQ VLEENKELENSLRNIQLPKVEIKKLKDGGLTFWYKMILPPQFDRSK KYPLLIQVYGGPCSQSVKSVFAVNWITYLASKEGIVIALVDGRGTA FQGDKFLHAVYRKLGVYEVEDQLTAVRKFIEMGFIDEERIAIWGW SYGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASIYSERFMGLPT KDDNLEHYKNSTVMARAEYFRNVDYLLIHGTADDNVHFQNSAQI AKALVNAQVDFQAMWYSDQNHGILSGRSQNHLYTHMTHFLKQC FSLSDGKKKKKKGHHHHHH	157
Cynomolgus FAP ectodomain+poly-lys-tag+his_6-tag	RPPRVHNSEENTMRALTLKDILNGTFSYKTFFPNWISGQEYLHQSA DNNIVLYNIETGQSYTILSNRTMKSVNASNYGLSPDRQFVYLESDY SKLWRYSYTATYYIYDLSNGEFVRGNELPRPIQYLCWSPVGSKLA YVYQNNIYLKQRPGDPPFQITFNGRENKIFNGIPDWVYEEEMLATK YALWWSPNGKFLAYAEFNDTDIPVIAYSYYGDEQYPRTINIPYPKA GAKNPFVRIFIIDTTYPAYVGPQEVPVPAMIASSDYYFSWLTWVTD ERVCLQWLKRVQNVSVLSICDFREDWQTWDCPKTQEHIEESRTG WAGGFFVSTPVFSYDAISYYKIFSDKDGYKHIHYIKDTVENAIQITS GKWEAINIFRVTQDSLFYSSNEFEDYPGRRNIYRISIGSYPPSKKCVT CHLRKERCQYYTASFSDYAKYYALVCYGPGIPISTLHDGRTDQEIK ILEENKELENALKNIQLPKEEIKKLEVDEITLWYKMILPPQFDRSKK YPLLIQVYGGPCSQSVRSVFAVNWISYLASKEGMVIALVDGRGTAF QGDKLLYAVYRKLGVYEVEDQITAVRKFIEMGFIDEKRIAIWGWS YGGYVSSLALASGTGLFKCGIAVAPVSSWEYYASVYTERFMGLPT KDDNLEHYKNSTVMARAEYFRNVDYLLIHGTADDNVHFQNSAQI AKALVNAQVDFQAMWYSDQNHGLSGLSTNHLYTHMTHFLKQCF SLSDGKKKKKKGHHHHHH	158

While there are shown and described presently preferred embodiments of the invention, it is to be distinctly understood that the invention is not limited thereto but may be otherwise variously embodied and practiced within the scope of the following claims.

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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) 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.

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.: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;

and wherein the antigen binding site specific for FAP comprises a variable heavy chain comprising an amino acid sequence selected from the group of: SEQ ID NO.:15 and SEQ ID NO.:39 ; and a variable light chain comprising an amino acid sequence selected from the group of SEQ ID NO.:16 and SEQ ID NO.:40.

3. The bispecific antibody of claim 1 or 2, 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;

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

4. The bispecific antibody of any of claims 1 to 3, wherein the antigen binding site specific for DR5

comprises 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; and the antigen binding site specific for FAP comprises a heavy chain variable region comprising an amino acid sequence of SEQ ID NO.:15 and a light chain variable region comprising an amino acid sequence of SEQ ID NO.:16.

5. The bispecific antibody of any of claims 1 to 4, comprising an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP.

6. The bispecific antibody of claim 5, wherein at least one of the Fab fragments is connected to the first or second subunit of the Fc domain via the light chain (VLCL) and at least one Fab fragment is connected to the first or second subunit of the Fc domain via the heavy chain (VHCH1).

7. The bispecific antibody of any of claims 1 to 6, comprising an Fc domain, at least one Fab fragment comprising the antigen binding site specific for DR5, and at least one Fab fragment comprising the antigen binding site specific for FAP wherein either the variable regions or the constant regions of the heavy and light chain of at least one Fab fragment are exchanged with each other.

8. The bispecific antibody of claim 7, comprising two Fab fragments comprising each an antigen binding site specific for DR5, and two Fab fragments comprising each an antigen binding site specific for FAP.

9. The bispecific antibody of claim 8, wherein the bispecific antibody is bivalent both for DR5 and FAP.

10. The bispecific antibody of claim 7, comprising two Fab fragments comprising each an antigen binding site specific for DR5, and one Fab fragment comprising an antigen binding site specific for FAP.

11. The bispecific antibody of claim 10, wherein the bispecific antibody is bivalent for DR5 and monovalent for FAP.

12. The bispecific antibody of claim 10, comprising one additional Fab fragment comprising an antigen binding site specific for DR5.

13. The bispecific antibody of claim 12, wherein the bispecific antibody is trivalent for DR5 and monovalent for FAP.

14. The bispecific antibody of claim 7, comprising one Fab fragment comprising an antigen binding site specific for DR5, and one Fab fragment comprising an antigen binding site specific for FAP.

15. The bispecific antibody of claim 14, wherein the bispecific antibody is monovalent for DR5 and monovalent for FAP.

16. The bispecific antibody of any of claims 7 to 15, wherein either the variable regions or the constant regions of the heavy and light chain of the Fab fragment(s) comprising an antigen binding site specific for FAP are exchanged with each other.

17. The bispecific antibody of any of claims 5 to 16, wherein at least one of the Fab fragments are connected to the Fc domain via a peptide linker.

18. The bispecific antibody of any of claims 5 to 17, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

19. The bispecific antibody of claim 18, wherein said one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 in the human IgG1 Fc domain.

20. The bispecific antibody of claim 19, 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 in the human IgG1 Fc domain.

21. The bispecific antibody of any of claims 5 to 20, wherein the Fc domain comprises a first heavy chain and a second heavy chain, wherein the first heavy chain comprises a first dimerization module and the second heavy chain comprises a second dimerization module allowing a heterodimerization of the two heavy chains.

22. The bispecific antibody of claim 21, wherein the first dimerization module comprises knobs and the second dimerization module comprises holes according to the knobs into holes strategy.

23. 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.

24. The antibody of claim 23, 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.

25. The antibody of claim 23, 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.: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.

26. The antibody of any of claims 23 to 25, 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.

27. A pharmaceutical composition comprising a bispecific antibody of claims 1 to 22 or the antibody of claims 23 to 26.

28. The bispecific antibody of claims 1 to 22 or the antibody of claims 23 to 26 for the treatment of cancer.

29. The bispecific antibody of claims 1 to 22 or the antibody of claims 23 to 26 for use as a medicament.

30. Use of the bispecific antibody of claims 1 to 22 or the antibody of claims 23 to 26 in the manufacture of a medicament for the treatment of cancer.

31. The use of claim 30, wherein the medicament is for treatment of pancreatic cancer or colorectal carcinoma.

32. Isolated polynucleotides encoding a heavy chain and a light chain of an antibody of claims 23 to 26.

33. An expression vector comprising a nucleic acid sequence of claim 32.

34. A prokaryotic or eukaryotic host cell comprising a vector according to claim 33.

35. A method of producing an antibody comprising culturing the host cell of claim 34 so that the antibody is produced.