SUMMARY OF THE INVENTION

In one aspect, the present invention provides a protein that binds an extracellular part of a membrane associated member of: the epidermal growth factor (EGF) receptor family or cMET; and an extracellular part of a membrane associated member of a WNT signaling pathway.

In another aspect, the present invention provides a bispecific antibody or a functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of a membrane associated member of the epidermal growth factor (EGF) receptor family or cMET and a variable domain that binds an extracellular part of a membrane associated member of a WNT-signaling pathway.

In yet another aspect, the present invention provides use of the protein or antibody disclosed herein or the bispecific antibody disclosed herein, or a functional part, derivative and/or analogue of the antibody in the manufacture of a medicament to treat cancer.

In yet another aspect, the present invention provides a cell system comprising a protein or antibody disclosed herein or the bispecific antibody disclosed herein, and a cell that expresses a membrane associated member of the epidermal growth factor (EGF) receptor family and that expresses a membrane associated member of the WNT pathway.

In yet another aspect, the present invention provides a method for inhibiting proliferation of a cell that expresses a membrane associated member of the epidermal growth factor (EGF) receptor family and that expresses a membrane associated member of the WNT pathway in a system permissive for proliferation of the cell, the method comprising providing the system with the protein or antibody disclosed herein or the bispecific antibody disclosed herein.

Disclosed is a protein that binds an extracellular part of a membrane associated member of the epidermal growth factor (EGF) receptor family and an extracellular part of a membrane associated member of a WNT signaling pathway. The protein is preferably an antibody, preferably a bispecific antibody or a functional part, derivative and/or analogue thereof.

The disclosure also provides a bispecific antibody or a functional part, derivative and/or analogue thereof that binds an extracellular part of a membrane associated member of the epidermal growth factor (EGF) receptor family and an extra-cellular part of a membrane associated member of a WNT-signaling pathway.

Also provided is a method for the treatment of an individual that has a cancer, the method comprising administering a protein of the disclosure or a bispecific antibody of the disclosure to the individual in need thereof.

The disclosure further provides a protein of the disclosure or a bispecific antibody of the disclosure, for use in the treatment of an individual that has cancer.

In one example the cancer is an EGF-receptor ligand responsive cancer that expresses a membrane associated member of the WNT pathway.

Further provided is a cell system comprising a protein of the disclosure or a bispecific antibody of the disclosure, and a cell that expresses a membrane associated member of the epidermal growth factor (EGF) receptor family and that expresses a membrane associated member of the WNT pathway. The cell is preferably an EGF-receptor ligand responsive cell that expresses a membrane associated member of the WNT pathway.

Also provided is a method for inhibiting growth of a cell that expresses a membrane associated member of the epidermal growth factor (EGF) receptor family and that expresses a membrane associated member of the WNT pathway in

a system permissive for growth of the cell, the method providing the system with a protein of the disclosure or a bispecific antibody of the disclosure. The cell is preferably an EGF-receptor ligand responsive cell that expresses a membrane associated member of the WNT pathway.

The disclosure provides an antibody that comprises a variable domain that can bind an epitope on an extracellular part of LGR5 which epitope is located within amino acid residues 21-118 of SEQ ID NO: 1 depicted in figure 39 of which amino acid residues D43; G44, M46, F67, G90, and F91 are involved in binding of the antibody to the epitope.

The antibody is preferably an antibody wherein one or more of the amino acid residue substitutions of D43A; G44A, M46A, F67A, G90A, and F91A reduces the binding of the antibody of LGR5.

The disclosure further provides an antibody that comprises a variable domain that can bind an epitope on an extracellular part of LGR5 which epitope is located within amino acid residues 21-118 of SEQ ID NO: 1 depicted in figure 39, and wherein the binding of the antibody to LGR5 reduced by one or more of the following amino acid residue substitutions D43A; G44A, M46A, F67A, G90A, and F91A.

The antibody is preferably an antibody wherein interaction of the antibody with LGR5 on an LGR5-expressing cell does not inhibit the binding of Rspondin 1 (RSPO 1) to LGR5 by more than 20%. The inhibition of binding of the antibody to LGR5 is preferably measured when the antibody and the RSPO are present in a molar ratio of 0,1 or less; preferably in a molar ratio of between 0,1 to 0,001, (inclusive), preferably 0,1 to 0,01 (inclusive).

The disclosure further provides an antibody that comprises a variable domain that can bind an epitope on LGR5 that is located within amino acid residues 21-118 of SEQ ID NO: 1 depicted in figure 39, and wherein interaction of the antibody with LGR5 on an LGR5-expressing cell does not inhibit the binding of

an RSPO to LGR5 by more than 20% when the antibody and the RSPO are present in a molar ratio of 0,1 or less; preferably in a molar ratio of between 0,1 to 0,001, (inclusive), preferably 0,1 to 0,01 (inclusive).

The epitope is preferably a conformational epitope. The epitope is preferably located within amino acid residues 40-95 of SEQ ID NO: 1 depicted in figure 39. The binding of the antibody to LGR5 is preferably reduced with one or more of the following amino acid residue substitutions D43A; G44A, M46A, F67A, G90A, and F91A. The antibody preferably further comprises a further variable domain that can bind a further protein. The further protein is preferably a membrane protein comprising an extracellular part. The further protein is preferably a membrane associated member of the epidermal growth factor (EGF) receptor family or cMET.

In a particularly preferred example the antibody is a bispecific antibody.

The bispecific antibody preferably comprises a variable domain that can bind said epitope on an extracellular part of LGR5 and a variable domain that can bind a further protein. As mentioned herein the further protein is preferably a membrane associated member of the EGFR receptor family or cMET. The variable domain that binds this further protein preferably binds an extracellular part of said further protein.

The disclosure further provides a bispecific antibody comprising a variable domain that can bind an epitope on an extracellular part of LGR5 and a variable domain that can bind a further protein, wherein the LGR5 epitope is located within amino acid residues 21-118 of SEQ ID NO: 1 depicted in figure 39 of which amino acid residues D43; G44, M46, F67, G90, and F91 are involved in binding of the antibody. The binding of the variable domain to LGR5 is preferably reduced with one or more of the following amino acid residue substitutions D43A; G44A, M46A, F67A, G90A, and F91A. The further protein is preferably a membrane protein comprising an extracellular part. The further protein is preferably a membrane associated member of the EGF receptor family or cMET.

The binding of the (bi)specific antibody to the membrane associated member of the EGF receptor family or cMET preferably reduces ligand-induced signaling in a cell that comprises said membrane associated member of the EGF receptor family or cMET.

The disclosure further provides an antibody that comprises a variable domain that can bind an epitope on an extracellular part of LGR5 which epitope is located within amino acid residues 21-118 of SEQ ID NO: 1 depicted in figure 39 and wherein the binding of the protein to LGR5 is reduced with one or more of the following amino acid residue substitutions D43A; G44A, M46A, F67A, G90A, and F91A.

Without being bound by theory it is believed that M46, F67, G90, and F91 of LGR5 as depicted in figure 39, are contact residues for the variable domain, i.e. the antigen-binding site of the variable domain that can bind the LGR epitope. That amino acid residue substitution D43A and G44A reduces the binding of the antibody can be due to the fact that these are contact residues, however, it is also possible that these amino acid residue substitution induce a (slight) modification of the conformation of the part of LGR that has one or more of the other contact residues (i.e. at positions 46, 67, 90 or 91) and that conformation change is such that antibody binding is reduced. The epitope is characterized by the mentioned amino acid substitutions. Whether an antibody binds the same epitope can be determined in various ways. In the examples a preferred method is described. The method utilizes a CHO cells. The CHO cells express LGR5 on the cell membrane, or on alanine substitution mutant, preferably a mutant comprising one or more of the substitutions M46A, F67A, G90A, or F91A. The test antibody is contacted with the CHO cells and binding of the antibody to the cells compared. A test antibody binds the epitope if it binds to LGR5 and to a lesser extent to an LGR5 with a M46A, F67A, G90A, or F91A substitution. Comparing binding with a panel of mutants each comprising one alanine residue substitution is preferred. Such binding studies are well known in the art. Often the panel comprises single alanine substitution mutants covering essentially all amino acid residues. For LGR5 the panel only needs to cover the extracellular part of the protein. Expression of a particular

mutant can be compromised but this is easily detected by one or more LGR5 antibodies that bind to different region(s). If expression is also reduced for these control antibodies the level or folding of the protein on the membrane is compromised for this particular mutant. Binding characteristics of the test antibody to the panel readily identifies whether the test antibodies exhibits reduced binding to mutants with a M46A, F67A, G90A, or F91A substitution and thus whether the test antibody is an antibody of the disclosure. Reduced binding to mutants with a M46A, F67A, G90A, or F91A substitution also identifies the epitope to be located within amino acid residues 21-118 of SEQ ID NO: 1 depicted in figure 39, the same applies to the location within amino acid residues 40-95 of SEQ ID NO: 1 depicted in figure 39. In a preferred example the panel includes a D43A substitution mutant; a G44A substitution mutant of both. The antibody with the VH sequence of the VH of MF5816 exhibits reduced binding to these substitution mutants.

In a preferred example the interaction of RSPO 1 with LGR5 on an LGR5-expressing cell does not inhibit the binding of the antibody to LGR5 by more than 20%. Inhibition of binding of the antibody to LGR5 is preferably measured under conditions wherein the antibody and the RSPO 1 are present in a molar ratio from of 0,1 or less; preferably in a molar ratio of between 0,1 to 0,001, (inclusive), preferably 0,1 to 0,01 (inclusive).

Inhibition of binding is preferably measured when the antibody and the RSPO 1 are present in a molar ratio from of 0,1 or less, preferably in a molar ratio of between 0,1 to 0,01 (inclusive). It was found that molar ratio's of antibody to RSPO of less than 0,001 can sometimes reduce the binding of the antibody to LGR5.

When herein molar ratio's of antibody to RSPO are mentioned it is preferred that the antibody is present in amounts that result in 40%-80% of the binding achieved when saturating amounts of the antibody are present.

The disclosure further provides an antibody that comprises a variable domain that can bind an epitope on an extracellular part of human EGFR of which amino acid residues I462; G465; K489; I491; N493; and C499 are involved in binding of the antibody to the epitope. The antibody is preferably an antibody characterized in that the binding of the antibody to EGFR is reduced with an EGFR wherein one or more of the amino acid residue substitutions selected from I462A; G465A; K489A; I491A; N493A; and C499A have been introduced.

The disclosure further provides an antibody that comprises a variable domain that can bind an epitope on an extracellular part of human EGFR which epitope is located within amino acid residues 420-480 of SEQ ID NO: 2 depicted in figure 40, and wherein the binding of the antibody to LGR5 is reduced by one or more of the following amino acid residue substitutions I462A; G465A; K489A; I491A; N493A; and C499A. The binding of the antibody to human EGFR interferes with the binding of EGF to the receptor. The epitope on EGFR is a conformational epitope.

The epitope is located within amino acid residues 420-480 of SEQ ID NO: 2 depicted in figure 40, preferably within 430-480 of SEQ ID NO: 2 depicted in figure 40; preferably within 438-469 of SEQ ID NO: 2 depicted in figure 40.

The antibody preferably comprises a further variable domain which further variable domain can bind a further protein. The further protein is preferably a membrane protein comprising an extracellular part. The further protein is preferably a membrane associated member WNT-pathway.

The antibody is typically a bispecific antibody.

The variable domain that binds human EGFR, is preferably a variable domain with a heavy chain variable region that comprises at least the CDR3 sequence of the VH of MF3755 as depicted in Figure 1 or a CDR3 sequence that differs in at most three, preferably in at most two, preferably in no more than one amino acid from a CDR3 sequence of the VH of MF3755 as depicted in Figure 1.

The variable domain that binds human EGFR, is preferably a variable domain with a heavy chain variable region that comprises at least the CDR1, CDR2 and CDR3 sequences of the VH of MF3755 as depicted in Figure 1; or the CDR1, CDR2 and CDR3 sequences of the VH of MF3755 as depicted in Figure 1 with at most three, preferably at most two, preferably at most one amino acid substitutions.

The variable domain that binds human EGFR, is preferably a variable domain with a heavy chain variable region that comprises the sequence of the VH chain of MF3755 as depicted in Figure 1; or the amino acid sequence of the VH chain of MF3755 depicted in Figure 1 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to the VH chain of MF3755.

The further protein is preferably LGR4; LGR5; LGR6; RNF43 or ZNRF3, preferably LGR5.

The disclosure further provides a bispecific antibody comprising a variable domain that can bind an epitope on an extracellular part of EGFR and a variable domain that can bind a further protein, wherein the EGFR epitope is located within amino acid residues 420-480 of SEQ ID NO: 2 depicted in figure 40, which amino acid residues I462; G465; K489; I491; N493; and C499 are involved in binding of the antibody to the epitope. The binding of the variable domain to EGFR is preferably reduced with one or more of the following amino acid residue substitutions I462A; G465A; K489A; I491A; N493A; and C499A. The further protein is preferably a membrane protein comprising an extracellular part, preferably a membrane associated member of the WNT-pathway, preferably LGR5.

Without being bound by theory it is believed that the contact residues of the epitope, i.e. where the variable domain contacts the human EGFR are likely I462; K489; I491; and N493. The amino acid residues G465 and C499 are likely indirectly involved in the binding of the antibody to EGFR, probably because

Head and Neck cancer; Melanoma; Testis cancer; Urothelial cancer; Renal cancer; Stomach cancer; or Carcinoid cancer.

14. The protein or antibody of any one of claims 1-7 or the bispecific antibody of any one of claims 8-10, for use of any one of claims 11-13, wherein the cancer is Colorectal cancer.

15. Use of the protein or antibody of any one of claims 1-7 or the bispecific antibody according to any one of claims 8-10, or a functional part, derivative and/or analogue of the antibody in the manufacture of a medicament to treat cancer.

16. A cell system comprising a protein or antibody of any one of claims 1-7 or the bispecific antibody according to any one of claims 8-10, and a cell that expresses a membrane associated member of the epidermal growth factor (EGF) receptor family and that expresses a membrane associated member of the WNT pathway.

17. A method for inhibiting proliferation of a cell that expresses a membrane associated member of the epidermal growth factor (EGF) receptor family and that expresses a membrane associated member of the WNT pathway in a system permissive for proliferation of the cell, the method comprising providing the system with the protein or antibody of any one of claims 1-7 or the bispecific antibody according to any one of claims 8-10.