

Title: Treatment of cancers with an antibody that binds LGR5 and EGFR

5 FIELD OF THE INVENTION

The disclosure relates to means and methods in the treatment of cancer. The disclosure in particular relates to a method of treating a cancer in an individual with an antibody that binds LGR5 and EGFR. The invention further relates to the combination for use in such methods and to the combination for use in the
10 manufacture of a medicament for the treatment of gastrointestinal cancer. Such antibodies are particularly useful in the treatment of gastric, esophageal, or gastro-esophageal-junction cancer.

BACKGROUND OF THE INVENTION

15 Traditionally, most cancer drug discovery has focused on agents that block essential cell functions and kill dividing cells via chemotherapy. However, chemotherapy rarely results in a complete cure. In most cases, the tumors in the patients stop growing or temporarily shrink (referred to as remission) only to start proliferating again, some times more rapidly (referred to as relapse), and become increasingly more difficult to
20 treat. More recently the focus of cancer drug development has moved away from broadly cytotoxic chemotherapy to targeted cytostatic therapies with less toxicity. Treatment of advanced cancer with targeted therapy that specifically inhibits signaling pathway components has been validated clinically in leukemia. However, in a majority of carcinomas, targeted approaches are still proving ineffective.

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Cancer is still a major cause of death in the world, in spite of the many advances that have been made in the treatment of the disease and the increased knowledge of the molecular events that lead to cancer. Gastric cancer, for example is the 5th most common diagnosed cancer worldwide and the 3rd mostly deadly. In 2018, an estimated
30 783,000 deaths were due to gastric cancer. Esophageal cancer is the 9th most common cancer and the 6th most common cause of cancer deaths. It has been reported that epidermal growth factor receptor (EGFR) is overexpressed in more than 30% of gastric adenocarcinoma (GAC) and esophageal adenocarcinoma (EAC) cases. However, an analysis reviewing of six different studies concluded that the addition of an anti-

EGFR agent to chemotherapy did not improve overall survival or progression free survival for patients with advanced/metastatic EAC, GAC or Gastro-esophageal junction adenocarcinoma (GEJAC) (Kim et al. 2017 Oncotarget. 2017 Nov 17; 8(58): 99033–99040). A need thus exists for cancer treatments, in particular for treatments
5 for gastric and esophageal cancer.

SUMMARY OF THE INVENTION

The disclosure provides the following preferred embodiments. However, the invention
10 is not limited to these embodiments.

In some embodiments the disclosure provides an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an
15 extracellular part of LGR5 for use in the treatment of cancer in a subject, wherein said use comprises providing the subject with a flat dose of 1500 mg of the antibody or functional part, derivative and/or analogue thereof. The disclosure further provides methods of treating cancer in a subject, comprises providing the subject in need thereof with a flat dose of 1500 mg of the
20 antibody or functional part, derivative and/or analogue thereof.

In some embodiments the disclosure provides an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an
25 extracellular part of LGR5 for use in the treatment of gastric, esophageal, or gastro-esophageal-junction cancer in a subject. The disclosure further provides methods of treating gastric, esophageal, or gastro-esophageal-junction cancer in a subject in a subject, comprises providing the subject in need thereof with the antibody or functional part, derivative and/or analogue thereof. Preferably
30 said use comprises providing the subject with a flat dose of 1500 mg of the antibody or functional part, derivative and/or analogue thereof.

In some embodiments, administrations of the therapeutic compound may be done weekly, biweekly or monthly. In some embodiments, the therapeutic compound is administered once every 2 weeks.

- 5 In some embodiments the disclosure provides an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 for use in the treatment of a gastric, esophageal, or gastro-esophageal-junction cancer in a Her2-negative subject. The disclosure
- 10 further provides methods of treating gastric, esophageal, or gastro-esophageal-junction cancer in a Her2-negative subject, comprises providing the subject in need thereof with the antibody or functional part, derivative and/or analogue thereof. Preferably said use comprises providing the subject with a flat dose of 1500 mg of the antibody or functional part, derivative and/or analogue thereof.
- 15 In some embodiments, administrations of the therapeutic compound to the Her2-negative subject may be done weekly, biweekly or monthly. Preferably, the therapeutic compound is administered once every 2 weeks.

- Preferably, the antibody or functional part, derivative and/or analogue thereof
- 20 is provided intravenously.

- Preferably, the cancer has a mutation in one or more genes selected from TP53, MLH1, PIK3CA, CDKN2A, UGT1A, UGT1A8, BRAF, PTEN, and KRAS, preferably wherein the cancer has a mutation in one or more gene selected
- 25 from TP53, MLH1, CDKN2A, UGT1A, UGT1A8, BRAF, and PTEN.
- Preferably, the cancer has one or more mutations selected from TP53 R196T; TP53 R342T; TP53 R248Q; MLH1 V384D; PIK3CA H1047R; CDKN2A W110T; UGT1A1 G71R; UGT1A8 G71R; and KRAS G12C.

Preferably, the cancer has a mutation in the gene coding for TP53, preferably wherein the mutation is R196T. Preferably, the cancer has a mutation in the gene coding for TP53, preferably wherein the mutation is R342T, and the cancer has a mutation in the gene coding for MLH1, preferably wherein the
5 mutation is V384D.

Preferably, the cancer has a mutation in the gene coding for TP53, preferably wherein the mutation is R248Q, the cancer has a mutation in the gene coding for PIK3CA, preferably wherein the mutation is H1047R, the cancer has a mutation in the gene coding for CDKN2A, preferably wherein the mutation is
10 W110T, the cancer has a mutation in the gene coding for UGT1A1, preferably wherein the mutation is G71R, and the cancer has a mutation in the gene coding for UGT1A8, preferably wherein the mutation is G71R.

Preferably, the cancer is esophageal cancer, preferably esophageal squamous
15 cell carcinoma (ESCC).

Preferably, the cancer has a mutation in the gene coding for BRAF. However, preferably the cancer does not have mutation V600E in BRAF, and wherein the cancer has a mutation in the gene encoding for PTEN. However, preferably
20 the cancer does not also have mutation R130Ter in PTEN.

Preferably, the cancer has a mutation in the gene coding for KRAS, preferably wherein the mutation is G12C, the cancer has a mutation in the gene coding for UGT1A1, preferably wherein the mutation is G71R, and the cancer has a
25 mutation in the gene coding for UGT1A8, preferably wherein the mutation is G71R.

Preferably, the cancer has a mutation in the gene coding for UGT1A1, preferably wherein the mutation is G71R, and the cancer has a mutation in
30 the gene coding for UGT1A8, preferably wherein the mutation is G71R.

Preferably, the cancer further has a mutation in PIK3CA, preferably wherein the mutation is E545K.

Preferably, the cancer is gastric cancer.

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- Preferably, the VH chain of the variable domain that binds EGFR comprises the amino acid sequence of VH chain MF3755 as depicted in figure 3; or the amino acid sequence of VH chain MF3755 as depicted in figure 3 having at most 15, preferably not more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 and preferably
- 10 having not more than 5, 4, 3, 2 or 1 amino acid modifications, including insertions, deletions, substitutions or a combination thereof with respect said VH; and wherein a VH chain of the variable domain that binds LGR5 comprises the amino acid sequence of VH chain MF5816 as depicted in figure 3; or the amino acid sequence of VH chain MF5816 as depicted in figure 3
- 15 having at most 15, preferably not more than 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 and preferably having not more than 5, 4, 3, 2 or 1 amino acid modifications, including insertions, deletions, substitutions or a combination thereof with respect said VH.
- 20 Preferably, the variable domain that binds LGR5 binds an epitope that is located within amino acid residues 21-118 of the human LGR5 sequence depicted in figure 1. Preferably, the amino acid residues at positions 43, 44, 46, 67, 90, and 91 of human LGR5 are involved in the binding of the LGR5 binding variable domain to LGR5. Preferably, the LGR5 binding variable domain binds
- 25 less to an LGR5 protein comprising one or more of the amino acid residue variations selected from 43A, 44A, 46A, 67A, 90A, and 91A.

- Preferably, the variable domain that binds EGFR binds an epitope that is located within amino acid residues 420-480 of the human EGFR sequence
- 30 depicted in figure 2. Preferably, the amino acid residues at positions I462,

G465, K489, I491, N493 and C499 of human EGFR are involved in the binding of the EGFR binding variable domain to EGFR. Preferably, the EGFR binding variable domain binds less to an EGFR protein comprising one or more of the amino acid residue substitutions selected from I462A, G465A, K489A, I491A,
5 N493A and C499A.

Preferably, the antibody is ADCC enhanced. Preferably, the antibody is afucosylated.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Human LGR5 sequence; Sequence ID NO: 1.

Figure 2 Human EGFR sequence; Sequence ID NO: 2.

15 Figure 3 (a) Amino acid sequences of heavy chain variable regions (Sequence ID Nos: 3-15) that together with a common light chain variable region such as the variable region of the human kappa light chain IgV κ 1 39*01/IGJ κ 1*01 form a variable domain that binds LGR5 and EGFR. The CDR and framework regions are indicated in figure 3b. Respective DNA sequence are indicated in figure 3c.

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Figure 4 a) Amino acid sequence of a common light chain amino acid sequence. b) Common light chain variable region DNA sequence and translation (IGKV1-39/jk1). c) Light chain constant region DNA sequence and translation. d) V-region IGKV1-39A; e) CDR1, CDR2 and CDR3 of a common light chain according to IMGT numbering.

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Figure 5 IgG heavy chains for the generation of bispecific molecules. a) CH1 region DNA sequence and translation. b) Hinge region DNA sequence and translation. c) CH2 region DNA sequence and translation. d) CH3 domain containing variations L351K and T366K (KK) DNA sequence and translation. e) CH3 domain containing
30 variations L351D and L368E (DE) DNA sequence and translation. Residue positions are according to EU numbering.

Figure 6 Data are showing mean tumor size in a) gastric PDX models and b) esophageal PDX models, error bars are SEM. Two-way ANOVA test was used to calculate statistical significance at given timepoint. ADC = adenocarcinoma. SCC = squamous-cell carcinoma. Gray areas represent the treatment period.

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DETAILED DESCRIPTION OF THE DISCLOSED EMBODIMENTS

In order that the present description may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed
10 description. Unless separately defined herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art, and conventional methods of immunology, protein chemistry, biochemistry, recombinant DNA techniques and pharmacology are employed.

15 As used herein, the singular forms "a", "an" and "the" include plural referents. Use of the term "comprising" "having" "including" as well as other forms, such as "comprise", "comprises", "comprised", "has", "have", "had", "include", "includes", and "included", is not limiting.

20 The term "antibody" as used herein means a proteinaceous molecule belonging to the immunoglobulin class of proteins, containing one or more domains that bind an epitope on an antigen, where such domains are or derived from or share sequence homology with the variable region of an antibody. Antibodies are typically made of basic structural units—each with two heavy chains and two light chains. An antibody
25 according to the present invention is not limited to any particular format or method of producing it.

A "bispecific antibody" is an antibody as described herein wherein one domain of the antibody binds to a first antigen whereas a second domain of the antibody binds to a
30 second antigen, wherein said first and second antigens are not identical, or where one domain binds a first epitope on an antigen, whereas a second domain binds to a second epitope on the antigen. The term "bispecific antibody" also encompasses antibodies wherein one heavy chain variable region/light chain variable region

(VH/VL) combination binds a first antigen or epitope on an antigen and a second VH/VL combination that binds a second antigen or epitope on the antigen. The term further includes antibodies wherein VH is capable of specifically recognizing a first antigen and the VL, paired with the VH in an immunoglobulin variable region, is
5 capable of specifically recognizing a second antigen. The resulting VH/VL pair will bind either antigen 1 or antigen 2. Such so called “two-in-one antibodies”, described in for instance WO 2008/027236, WO 2010/108127 and Schaefer et al (Cancer Cell 20, 472-486, October 2011). A bispecific antibody according to the present invention is not limited to any particular bispecific format or method of producing it.

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The term ‘common light chain’ as used herein refers to the two light chains (or the VL part thereof) in the bispecific antibody. The two light chains (or the VL part thereof) may be identical or have some amino acid sequence differences while the binding specificity of the full length antibody is not affected. The terms ‘common light chain’,
15 ‘common VL’, ‘single light chain’, ‘single VL’, with or without the addition of the term ‘rearranged’ are all used herein interchangeably. “Common” also refers to functional equivalents of the light chain of which the amino acid sequence is not identical. Many variants of said light chain exist wherein mutations (deletions, substitutions, insertions and/or additions) are present that do not influence the formation of
20 functional binding regions. The light chain of the present invention can also be a light chain as specified herein, having from 0 to 10, preferably from 0 to 5 amino acid insertions, deletions, substitutions, additions or a combination thereof. It is for instance within the scope of the definition of common light chains as used herein, to prepare or find light chains that are not identical but still functionally equivalent,
25 e.g., by introducing and testing conservative amino acid changes, changes of amino acids in regions that do not or only partly contribute to binding specificity when paired with the heavy chain, and the like.

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As used herein, “to comprise” and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition the verb “to consist” may be replaced by “to consist essentially of” meaning that a compound or adjunct compound as defined herein may

comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristic of the invention.

The term 'full length IgG' or 'full length antibody' according to the invention is defined as comprising an essentially complete IgG, which however does not necessarily have all functions of an intact IgG. For the avoidance of doubt, a full length IgG contains two heavy and two light chains. Each chain contains constant (C) and variable (V) regions, which can be broken down into domains designated CH1, CH2, CH3, VH, and CL, VL. An IgG antibody binds to antigen via the variable region domains contained in the Fab portion, and after binding can interact with molecules and cells of the immune system through the constant domains, mostly through the Fc portion. Full length antibodies according to the invention encompass IgG molecules wherein variations may be present that provide desired characteristics. Full length IgG should not have deletions of substantial portions of any of the regions. However, IgG molecules wherein one or several amino acid residues are deleted, without essentially altering the binding characteristics of the resulting IgG molecule, are embraced within the term "full length IgG". For instance, such IgG molecules can have a deletion of between 1 and 10 amino acid residues, preferably in non-CDR regions, wherein the deleted amino acids are not essential for the antigen binding specificity of the IgG.

A "derivative of an antibody" is a protein that but for the CDR regions deviates from the amino acid sequence of a natural antibody in at most 20 amino acids. A derivative of an antibody as disclosed herein is an antibody that deviates from said amino acid sequence in at most 20 amino acids.

"Percent (%) identity" as referring to nucleic acid or amino acid sequences herein is defined as the percentage of residues in a candidate sequence that are identical with the residues in a selected sequence, after aligning the sequences for optimal comparison purposes. The percent sequence identity comparing nucleic acid sequences is determined using the AlignX application of the Vector NTI Advance® 11.5.2 software using the default settings, which employ a modified ClustalW algorithm (Thompson, J.D., Higgins, D.G., and Gibson T.J., (1994) Nuc. Acid Res. 22(22): 4673-

- 4680), the swgapdnamt score matrix, a gap opening penalty of 15 and a gap extension penalty of 6.66. Amino acid sequences are aligned with the AlignX application of the Vector NTI Advance® 11.5.2 software using default settings, which employ a modified ClustalW algorithm (Thompson, J.D., Higgins, D.G., and Gibson T.J., (1994) Nuc. Acid Res. 22(22): 4673-4680), the blosum62mt2 score matrix, a gap opening penalty of 10 and a gap extension penalty of 0.1.

- As an antibody typically recognizes an epitope of an antigen, and such an epitope may be present in other compounds as well, antibodies according to the present invention that “specifically recognize” an antigen, for example, EGFR or LGR5, may recognize other compounds as well, if such other compounds contain the same kind of epitope. Hence, the terms “specifically recognizes” with respect to an antigen and antibody interaction does not exclude binding of the antibodies to other compounds that contain the same kind of epitope.

- The term “epitope” or “antigenic determinant” refers to a site on an antigen to which an immunoglobulin or antibody specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein (so-called linear and conformational epitopes). Epitopes formed from contiguous, linear amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding, conformation are typically lost on treatment with denaturing solvents. An epitope may typically include 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation.

- As used herein, the terms “subject” and “patient” are used interchangeably and refer to a mammal such as a human, mouse, rat, hamster, guinea pig, rabbit, cat, dog, monkey, cow, horse, pig and the like (e.g., a patient, such as a human patient, having cancer).

- The terms “treat,” “treating,” and “treatment,” as used herein, refer to any type of intervention or process performed on, or administering an active agent or combination of active agents to the subject with the objective of reversing, alleviating, ameliorating, inhibiting, or slowing down or preventing the progression, development,

severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

As used herein, "effective treatment" or "positive therapeutic response" refers to a treatment producing a beneficial effect, e.g., amelioration of at least one symptom of a disease or disorder, e.g., cancer. A beneficial effect can take the form of an improvement over baseline, including an improvement over a measurement or observation made prior to initiation of therapy according to the method. For example, a beneficial effect can take the form of slowing, stabilizing, stopping or reversing the progression of a cancer in a subject at any clinical stage, as evidenced by a decrease or elimination of a clinical or diagnostic symptom of the disease, or of a marker of cancer. Effective treatment may, for example, decrease in tumor size, decrease the presence of circulating tumor cells, reduce or prevent metastases of a tumor, slow or arrest tumor growth and/or prevent or delay tumor recurrence or relapse.

The term "effective amount" or "therapeutically effective amount" refer to an amount of an agent or combination of agents that provides the desired biological, therapeutic, and/or prophylactic result. That result can be reduction, amelioration, palliation, lessening, delaying, and/or alleviation of one or more of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. In some embodiments, an effective amount is an amount sufficient to delay tumor development. In some embodiments, an effective amount is an amount sufficient to prevent or delay tumor recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and may stop cancer cell infiltration into peripheral organs; (iv) inhibit tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer. In one example, an "effective amount" is the amount of an EGFR/LGR5 antibody to affect a decrease in a cancer (for example a decrease in the number of cancer cells); slowing of progression of a cancer, or prevent regrowth or recurrence of the cancer.

The present disclosure provides an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 for use in the treatment of cancer. The words cancer and tumor are used herein and typically both
5 refer to cancer, unless otherwise specifically stated.

Epidermal growth factor (EGF) receptor (EGFR, ErbB1, or HER1) is a member of a family of four receptor tyrosine kinases (RTKs), named Her- or cErbB-1, -2, -3 and -4. EGFR is known under various synonyms, the most common of which is EGFR. EGFR
10 has an extracellular domain (ECD) that is composed of four sub-domains, two of which are involved in ligand binding and two of which are involved in homo-dimerisation and hetero-dimerisation. EGFR integrates extracellular signals from a variety of ligands to yield diverse intracellular responses. A major signal transduction pathway activated by EGFR is composed of the Ras-mitogen-activated protein kinase
15 (MAPK) mitogenic signaling cascade. Activation of this pathway is initiated by the recruitment of Grb2 to tyrosine phosphorylated EGFR. This leads to activation of Ras through the Grb2-bound Ras-guanine nucleotide exchange factor Son of Sevenless (SOS). In addition, the PI3-kinase-Akt signal transduction pathway is also activated by EGFR, although this activation is much stronger in case there is co-expression of
20 ErbB-3 (HER3). The EGFR is implicated in several human epithelial malignancies, notably cancers of the breast, bladder, non-small cell lung cancer lung, colon, ovarian head and neck and brain. Activating mutations in the gene have been found, as well as over-expression of the receptor and of its ligands, giving rise to autocrine activation loops. This RTK has therefore been extensively used as target for cancer therapy.
25 Both small-molecule inhibitors targeting the RTK and monoclonal antibodies (mAbs) directed to the extracellular ligand-binding domains have been developed and have shown hitherto several clinical successes, albeit mostly for a select group of patients. The database accession number for the human EGFR protein and the gene encoding it is GenBank NM_005228.3. This accession number is primarily given to provide a
30 further method of identification of EGFR protein as a target, the actual sequence of the EGFR protein bound by an antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like.

Where reference herein is made to EGFR, the reference refers to human EGFR unless otherwise stated. The variable domain antigen-binding site that binds EGFR, binds EGFR and a variety of variants thereof such as those expressed on some EGFR positive tumors.

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The term "LGR" refers to the family of proteins known as Leucine-rich repeat-containing G-protein coupled receptors. Several members of the family are known to be involved in the WNT signaling pathway, of note LGR4; LGR5 and LGR6.

- 10 LGR5 is Leucine-Rich Repeat Containing G Protein-Coupled Receptor 5. Alternative names for the gene or protein are Leucine-Rich Repeat Containing G Protein-Coupled Receptor 5; Leucine-Rich Repeat-Containing G Protein-Coupled Receptor 5; G-Protein Coupled Receptor HG38; G-Protein Coupled Receptor 49; G-Protein Coupled Receptor 67; GPR67; GPR49; Orphan G Protein-Coupled Receptor HG38; G Protein-Coupled
- 15 Receptor 49; GPR49; HG38 and FEX. A protein or antibody of the invention that binds LGR5, binds human LGR5. The LGR5 binding protein or antibody of the invention may, due to sequence and tertiary structure similarity between human and other mammalian orthologs, also bind such an ortholog but not necessarily so. Database accession numbers for the human LGR5 protein and the gene encoding it
- 20 are (NC_000012.12; NT_029419.13; NC_018923.2; NP_001264155.1; NP_001264156.1; NP_003658.1). The accession numbers are primarily given to provide a further method of identification of LGR5 as a target, the actual sequence of the LGR5 protein bound may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. The LGR5 antigen binding site binds
- 25 LGR5 and a variety of variants thereof, such as those expressed by some LGR5 positive tumor cells.

- In some embodiments, the cancer is a gastrointestinal cancer such as colorectal cancer. Preferably, the cancer is gastric, esophageal, or gastro-esophageal-junction
- 30 cancer. Gastric cancer (also referred to as stomach cancer) is a cancer that develops from the lining of the stomach and in particular the mucus-producing glandular cells found therein. Such a cancer is also referred to as adenocarcinoma, or in this case gastric adenocarcinoma as is develops from the lining of the stomach. In a preferred

embodiment, the cancer is thus a gastric adenocarcinoma or cancer that develops from the lining of the stomach which is used interchangeably herein. Esophageal cancer is cancer that develops from the esophagus. The two main subtypes are ESCC (esophageal squamous-cell carcinoma) and EAC (esophageal adenocarcinoma).

- 5 Gastro-esophageal-junction cancer (also known as gastro-esophageal junction adenocarcinoma) arises from the gastro-esophageal junction.

- In some embodiments, the cancer expresses LGR5 and/or expresses EGFR. As used herein, a cancer expresses LGR5 if the cancer comprises cells that express LGR5. A
10 cell which expresses LGR5 comprises detectable levels of RNA that codes for LGR5. As used herein, a cancer expresses EGFR if the cancer comprises cells that express EGFR. A cell which expresses EGFR comprises detectable levels of RNA that codes for LGR5. Expression can often also be detected by incubating the cell with an antibody
15 that binds to LGR5 or EGFR. However, some cells do not express the protein high enough for such an antibody test. In such cases mRNA or other forms of nucleic acid sequence detection is preferred.

- In some embodiments the disclosure provides an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an
20 extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 for use in the treatment of a gastric, esophageal, or gastro-esophageal-junction cancer in a subject with a Her2 status selected from being Her2 positive, Her2 high, Her2 3+, Her2 2+, Her2 1+, Her2 0 or Her2-negative subject. Preferably, the subject is Her2 negative. The disclosure further provides methods of treating gastric,
25 esophageal, or gastro-esophageal-junction cancer in a Her2-negative subject, comprises providing the subject in need thereof with the antibody or functional part, derivative and/or analogue thereof. Preferably said use comprises providing the subject with a flat dose of 1500 mg of the antibody or functional part, derivative and/or analogue thereof. In some embodiments, administrations of the therapeutic
30 compound to the Her2-negative subject may be done weekly, biweekly or monthly. Preferably, the therapeutic compound is administered once every 2 weeks.

Methods for determining an expression of human epidermal growth factor receptor 2 (HER2) of a subject are well known in the art. For instance, the expression level of Her2 can be established using immunohistochemistry (IHC) or (fluorescence) in-situ hybridization (ISH), which allows identification of a Her2-status, including

5 identification of Her2 negative subject. IHC or ISH are both well-defined and standard procedures routinely used for establishing Her2 status in human subjects. Reference herein is made for instance to ASCO/CAP guidelines according to Bartley et al., (HER2 Testing and Clinical Decision Making in Gastroesophageal

10 Adenocarcinoma. Arch Pathol Lab Med. 2016;140:1345–1363). For instance, using the anti-HER-2/neu antibody (clone 4B5) allows for semi-quantitative detection of HER-2 antigen in sections of FFPE gastric/gastroesophageal adenocarcinoma, gastric, esophageal, or gastro-esophageal-junction cancer using IHC. Staining and scoring is performed according to consensus guidelines for this cancer type. Such an IHC test typically gives a score of 0 to 3+ that measures the amount of HER2 receptor protein

15 on the surface of cells in a cancer tissue sample. Based on the IHC score, a patient can be classified as being Her2 negative, such as when a score of 0 or 1+ is measured. In case an ISH test is used to establish Her2 expression, such as using the HER2 probe (17q11.2-q12) and centromere 17 probe (Cen 17), the diagnosis is either “positive” or “negative”, sometimes also reported as “zero” for HER2. A method of treatment of the

20 present disclosure is a subject who is preferably Her2-negative as established by IHC and/or ISH.

With a Her2 negative subject herein is meant a subject that has a cancer, a cancer cell or a tumor, that is Her2-negative. Her2 status may be determined in accordance with

25 IHC and/or ISH as described above.

Preferably, in some embodiments, the treatment with the antibody or functional part, derivative and/or analogue thereof is preceded by a step of diagnosing the subject for Her2 status. Preferably, in some embodiments, subjects having Her2-negative status

30 are selected for treatment. Preferably, in some embodiments, the treatment of a subject is preceded by a step of diagnosing a subject of having a Her2 negative gastric, esophageal, or gastro-esophageal-junction cancer. Such cancer treated by the method

of the present disclosure includes gastric adenocarcinoma and esophageal cancer having squamous cell carcinoma histology.

Said Her2 negative diagnosis preferably involves ISH or IHC testing of Her2 status.

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Preferably, in some embodiments, the treatment of a Her2 negative subject is preceded by a step of screening a subject of having a Her2 negative gastric, esophageal, or gastro-esophageal-junction cancer. Such cancer in particular is an adenocarcinoma. Said screening preferably involves ISH or IHC testing of Her2

10 status.

Cancers, such as gastric, esophageal, or gastro-esophageal-junction cancer, can be related to the presence of mutations. Such mutations include mutations in known oncogenes such as PIK3CA, KRAS and BRAF. Oncogenic mutations are generally

15 described as activating mutations or mutations which result in new functions.

Another type of cancer mutation involves tumor suppressor genes, such as TP53, MLH1, CDKN2A, and PTEN. Mutations in tumor suppressor genes are generally inactivating.

20 TP53 encodes a transcription factor that regulates a number of activities include stress response and cell proliferation. Mutations in TP53 are associated with various cancers and are estimated to occur in more than 50% of human cancers, including gastric and esophageal cancer. In particular, the TP53 R248Q mutation was shown to be associated with cancer, including gastric and esophageal cancer (Pitolli et al. Int. J. Mol. Sci.2019 20:6241). Nonsense mutations at positions R196 and R342 have been

25 identified in a number of tumors such as from breast and esophagus; and ovary, prostate, breast, pancreas, stomach, colon/rectum, lung, esophagus, bone; respectively (Priestly et al. Nature 2019 575: 210-216). In some embodiments, the therapeutic compounds disclosed herein are useful for treating a cancer having a TP53 mutation,

30 in particular a mutation that results in reduced TP53 expression or activity.

MLH1 (MutL homolog 1) encodes a protein involved in DNA mismatch repair and is a known tumor suppressor gene. Mutations in MLH1 are associated with various

cancers including gastrointestinal cancer. Low levels of MLH1 are also associated with esophageal cancer patients having a family history of esophageal cancer (Chang et al. *Oncol Lett.* 2015 9:430-436) and MLH1 is mutated in 1.39% of malignant esophageal neoplasm patients (The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discovery.* 2017;7(8):818-831. Dataset Version 6). In particular, the MLH1 V384D mutation was shown to be associated with cancers, e.g., colorectal cancer (Ohsawa et al. *Molecular Medicine Reports* 2009 2:887-891). In some embodiments, the therapeutic compounds disclosed herein are useful for treating a cancer having a MLH1 mutation, in particular a mutation which results in reduced MLH1 expression or activity.

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) encodes the 110 kDa catalytic subunit of PI3K (phosphatidylinositol 3-kinase). Mutations in PIK3CA are associated with various cancers include gastrointestinal cancer. As reported by the American Association for Cancer Research, PIK3CA is mutated in 12.75% of malignant solid tumor patients. In particular, the PIK3CA H1047R mutation is present in 2.91% of all malignant solid tumor patients and the PIK3CA E545K present in 2.55% of all malignant solid tumor patients (see, The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discovery.* 2017;7(8):818-831. Dataset Version 6.) In some embodiments, the therapeutic compounds disclosed herein are useful for treating a cancer having a PIK3CA mutation, in particular an oncogenic mutation in PIK2CA.

CDKN2A (Cyclin-dependent kinase inhibitor 2A) encodes a protein that inhibits CDK4 and ARF. As reported by the American Association for Cancer Research, CDKN2A is mutated in 22.21% of esophageal carcinoma patients, 28.7% of esophageal squamous cell carcinoma patients, and 6.08% of gastric adenocarcinoma patients. In particular, the CDKN2A W110Ter mutation is present in around 0.11% of cancer patients. (The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. *Cancer Discovery.* 2017;7(8):818-831. Dataset Version 6). In some embodiments, the therapeutic

compounds disclosed herein are useful for treating a cancer having a CDKN2A mutation, in particular a mutation which results in reduced CDKN2A expression or activity.

- 5 PTEN (phosphatase and tensin homolog) encodes for a phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase. As reported by the American Association for Cancer Research, PTEN is mutated in 6.28% of cancer patients, 3.41% of gastric adenocarcinoma patients, 2.37% of esophageal carcinoma patients, and in 2.22% of esophageal adenocarcinoma patients. In particular, the PTEN R130Ter mutation
10 (wherein Ter refers to a termination/stop codon) is present in 0.21% of all colorectal carcinoma patients (The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. Cancer Discovery. 2017;7(8):818-831. Dataset Version 6). In some embodiments, the therapeutic compounds disclosed herein are useful for treating a cancer having a PTEN mutation,
15 in particular a mutation which results in reduced PTEN expression or activity.

- BRAF encodes serine/threonine-protein kinase B-Raf, which is involved in growth signaling. As reported by the American Association for Cancer Research, BRAF is mutated in 1.91% of gastric carcinoma patients and in in 1.93% of gastric
20 adenocarcinoma patients. In particular, the BRAF V600E mutation is present in 2.72% of cancer patients (see, The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. Cancer Discovery. 2017;7(8):818-831. Dataset Version 6.). In some embodiments, the therapeutic compounds disclosed herein are useful for treating a
25 BRAF mutation, in particular an oncogenic mutation in BRAF. However, in some embodiments, the therapeutic compounds disclosed herein are useful for treating a gastric cancer that does not have BRAF mutation V600E.

- KRAS (Kirsten RAt Sarcoma) encodes a protein that is party of the RAS/MAPK
30 pathway. As reported by the American Association for Cancer Research, KRAS is mutated in 14.7% of malignant solid tumor patients with KRAS G12C present in 2.28% of all malignant solid tumor patients (see, The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an

international consortium. Cancer Discovery. 2017;7(8):818-831. Dataset Version 6.). In some embodiments, the therapeutic compounds disclosed herein are useful for treating a cancer having a KRAS mutation, in particular an oncogenic mutation in KRAS.

5

UGT1A1 (uridine diphosphateglucuronosyl transferase 1A1) and UGT1A8 (uridine diphosphateglucuronosyl transferase 1A8) encode enzymes of the glucuronidation pathway. Several polymorphisms which reduce enzyme activity are known to affect the metabolism and effect of irinotecan. For example, the UGT1A1*6 allele (G71R polymorphism) having an allele frequency of around 0.13% in Chinese, Korean, and Japanese populations and the UGT1A1*28 allele (dinucleotide repeat polymorphism in the TATA sequence of the promoter region) are risk factors for irinotecan induced neutropenia. In some embodiments, the therapeutic compounds disclosed herein are useful for treating a cancer having a UGT1A1 and/or UGT1A8 mutation, in particular a mutation that results in reduced expression or activity of UGT1A1 and/or UGT1A8.

10

ATM (Ataxia Telangiectasia Mutated), is a member of the serine-threonine kinase family and coordinates cellular responses to DNA damage through activation of distinct DNA repair and signaling pathways. ATM germline mutations are associated with ataxia telangiectasia and ATM somatic mutations are commonly observed in endometrial, colon, pancreatic, breast cancers and urothelial cancer.

20

In preferred embodiments, the disclosure provides methods for treating a cancer having a mutation in the gene encoding for TP53, MLH1, PIK3CA, CDKN2A, UGT1A, UGT1A8, BRAF, PTEN, and KRAS. Preferably, the cancer has one or more mutations selected from TP53 R196T; TP53 R342T; TP53 R248Q; MLH1 V384D; PIK3CA H1047R; PIK3CA E545K; CDKN2A W110T; UGT1A1 G71R; UGT1A8 G71R; and KRAS G12C. In some embodiments, the cancer is wild-type for KRAS. Alternatively, the disclosure provides methods for treating a cancer having a mutation in the gene encoding for ATM, in particular mutation W57T. In particular, the disclosure provides methods for treating esophageal cancer, in particular ESCC, having a mutation in the gene encoding for ATM, in particular mutation W57T.

25

30

In some embodiments the cancer has a mutation in the gene coding for TP53, preferably wherein the mutation is R342T, and the cancer has a mutation in the gene coding for MLH1, preferably wherein the mutation is V384D.

- 5 In some embodiments the cancer has a mutation in the gene coding for TP53, preferably wherein the mutation is R248Q, the cancer has a mutation in the gene coding for PIK3CA, preferably wherein the mutation is H1047R, the cancer has a mutation in the gene coding for CDKN2A, preferably wherein the mutation is W110T, the cancer has a mutation in the gene coding for UGT1A1, preferably wherein the mutation is G71R, and the cancer has a mutation in the gene coding for UGT1A8, preferably wherein the mutation is G71R. Preferably, the cancer is esophageal cancer, preferably esophageal squamous cell carcinoma (ESCC).

In some embodiments the cancer has a mutation in the gene coding for BRAF.

- 15 However, the cancer preferably does not have mutation V600E in the gene coding for BRAF, and preferably does not have mutation R130Ter in the gene encoding for PTEN. In some embodiments the cancer has a mutation in the gene coding for KRAS, preferably wherein the mutation is G12C, the cancer has a mutation in the gene coding for UGT1A1, preferably wherein the mutation is G71R, and the cancer has a mutation in the gene coding for UGT1A8, preferably wherein the mutation is G71R. In some embodiments the cancer has a mutation in the gene coding for UGT1A1, preferably wherein the mutation is G71R, and the cancer has a mutation in the gene coding for UGT1A8, preferably wherein the mutation is G71R. In some embodiments the cancer has a mutation in PIK3CA, preferably wherein the mutation is E545K.
- 25 Preferably, the cancer is gastric cancer.

- An antibody or a functional part, derivative and/or analogue thereof as described herein comprises a variable domain that binds an extracellular part of the epidermal growth factor (EGF) receptor and a variable domain that binds LGR5. The EGFR is preferably a human EGFR. The LGR5 is preferably a human LGR5. The antibody or a functional part, derivative and/or analogue thereof as described herein comprises a variable domain that binds an extracellular part of a human epidermal growth factor (EGF) receptor and a variable domain that binds a human LGR5.
- 30

Preferably the antibody or a functional part, derivative and/or analogue thereof as described herein comprises a variable domain that binds an extracellular part of the epidermal growth factor (EGF) receptor and interferes with the binding of EGF to the receptor and a variable domain that binds LGR5 wherein interaction of the antibody with LGR5 on an LGR5-expressing cell does not block the binding of an Rspodin (RSPO) to LGR5. Methods for determining whether an antibody blocks or does not block the binding of an Rspodin to LGR5 are described in WO2017069528, which is hereby incorporated by reference.

10

Where herein accession numbers or alternative names of proteins/genes are given, they are primarily given to provide a further method of identification of the mentioned protein as a target, the actual sequence of the target protein bound by an antibody of the invention may vary, for instance because of a mutation and/or alternative splicing in the encoding gene such as those occurring in some cancers or the like. The target protein is bound by the antibody as long as the epitope is present in the protein and the epitope is accessible to the antibody.

An antibody or a functional part, derivative and/or analogue thereof as described herein preferably interferes with the binding of a ligand for EGFR to EGFR. The term “interferes with binding” as used herein means that binding of the antibody or a functional part, derivative and/or analogue thereof to the EGFR competes with the ligand for binding to EGF receptor. The antibody or a functional part, derivative and/or analogue thereof may diminish ligand binding, displace ligand when this is already bound to the EGF receptor or it may, for instance through steric hindrance, at least partially prevent that ligand can bind to the EGF receptor.

An EGFR antibody as disclosed herein preferably inhibits respectively EGFR ligand-induced signaling, measured as ligand-induced growth of BxPC3 cells (ATCC CRL-1687) or BxPC3-luc2 cells (Perkin Elmer 125058) or ligand-induced cell death of A431 cells (ATCC CRL-1555). EGFR can bind a number of ligands and stimulate growth of the mentioned BxPC3 cells or BxPC3-luc2 cells. In the presence of an EGFR ligand the growth of BxPC3 or BxPC3-luc2 cells is stimulated. EGFR ligand-induced growth

of BxPC3 cells can be measured by comparing the growth of the cells in the absence and presence of the ligand. The preferred EGFR ligand for measuring EGFR ligand-induced growth of BxPC3 or BxPC3-luc2 cells is EGF. The ligand-induced growth is preferably measured using saturating amounts of ligand. In a preferred embodiment

5 EGF is used in an amount of 100ng/ml of culture medium. EGF is preferably the EGF R&D systems, cat. nr. 396-HB and 236-EG (see also WO2017/069628; which is incorporated by reference herein).

An EGFR antibody as disclosed herein preferably inhibits EGFR ligand induced

10 growth of BxPC3 cells (ATCC CRL-1687) or BxPC3-luc2 cells (Perkin Elmer 125058). EGFR can bind a number of ligands and stimulate growth of the mentioned BxPC3 cells or BxPC3-luc2 cells. In the presence of a ligand the growth of BxPC3 or BxPC3-luc2 cells is stimulated. EGFR ligand-induced growth of BxPC3 cells can be measured by comparing the growth of the cells in the absence and presence of the ligand. The

15 preferred EGFR ligand for measuring EGFR ligand-induced growth of BxPC3 or BxPC3-luc2 cells is EGF. The ligand-induced growth is preferably measured using saturating amounts of ligand. In a preferred embodiment EGF is used in an amount of 100ng/ml of culture medium. EGF is preferably the EGF of R&D systems, cat. nr. 396-HB and 236-EG (see also WO2017/069628; which is incorporated by reference

20 herein).

For the avoidance of doubt the reference to the growth of a cell as used herein refers to a change in the number of cells. Inhibition of growth refers to a reduction in the number of cells that would otherwise have been obtained. Increase in growth refers to

25 an increase in the number of cells that would otherwise have been obtained. The growth of a cell typically refers to the proliferation of the cell.

Whether an antibody as described herein inhibits signaling or inhibits growth in a multispecific format is preferably determined by the method as described herein

30 above using a monospecific monovalent or monospecific bivalent version of the antibody. Such an antibody preferably has binding sites for the receptor of which signaling is to be determined. A monospecific monovalent antibody can have a variable domain with an irrelevant binding specificity such as tetanus toxoid

specificity. A preferred antibody is a bivalent monospecific antibody wherein the antigen binding variable domains consist of variable domains that bind the EGF-receptor family member.

- 5 In its Biclomics® antibody program, Merus has developed multispecific antibodies that target EGFR and LGR5 (Leucine -rich repeat containing G protein-coupled receptor). The efficacy of such multispecific antibodies has been assessed in vitro and in vivo using patient-derived CRC organoids and mice PDX models, respectively (see, e.g., WO2017/069628; which is incorporated by reference herein). Multispecific antibodies
- 10 that target EGFR and LGR5 were shown to inhibit tumor growth. The potency of such inhibitory antibodies was shown to be correlated with the levels of LGR5 RNA expression by cells from the cancer. Multispecific antibodies that target EGFR and LGR5 as described in WO2017/069628 are particularly preferred.
- 15 An antibody or a functional part, derivative and/or analogue thereof as described herein comprises a variable domain that binds an extracellular part of LGR5. The variable domain that binds an extracellular part of LGR5 preferably binds an epitope that is located within amino acid residues 21-118 of the sequence of Figure 1 of which amino acid residues D43; G44, M46, F67, R90, and F91 are involved in binding of the
- 20 antibody to the epitope.

The LGR5 variable domain is preferably a variable domain wherein one or more of the amino acid residue substitutions in LGR5 of D43A; G44A, M46A, F67A, R90A, and F91A reduces the binding of the variable domain to LGR5.

- 25 The epitope on an extracellular part of LGR5 is preferably located within amino acid residues 21-118 of the sequence of Figure 1. It is preferably an epitope wherein the binding of the LGR5 variable domain to LGR5 is reduced by one or more of the following amino acid residue substitutions D43A; G44A, M46A, F67A, R90A, and
- 30 F91A in LGR5.

The disclosure further provides an antibody with a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of

LGR5 wherein the LGR5 variable domain binds an epitope on LGR5 that is located within amino acid residues 21-118 of the sequence of Figure. 1

5 The epitope on LGR5 is preferably a conformational epitope. The epitope is preferably located within amino acid residues 40-95 of the sequence of Figure 1. 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, R90A, and F91A.

10 Without being bound by theory it is believed that M46, F67, R90, and F91 of LGR5 as depicted in Figure 1, are contact residues for a variable domain as indicated herein above, i.e. the antigen-binding site of a variable domain that binds the LGR5 epitope. That amino acid residue substitution D43A and G44A reduces the binding of an antibody can be due to the fact that these are also contact residues, however, it is also possible that these amino acid residue substitution induce a (slight) modification of
15 the conformation of the part of LGR5 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 an exemplary method, CHO cells express LGR5 on the cell
20 membrane, or on alanine substitution mutant, preferably a mutant comprising one or more of the substitutions M46A, F67A, R90A, or F91A. A 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, R90A, or F91A substitution. Comparing binding with a panel of mutants each
25 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 and a part that warrants association with the cell membrane of course, when cell are used. Expression of a particular mutant can be
30 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, R90A, or F91A substitution and thus whether the test antibody is an antibody of the invention. Reduced binding to mutants with a M46A, F67A, R90A, or F91A substitution also identifies the epitope to be located within amino acid residues 21-118 of the sequence of Figure 1. In a preferred embodiment 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.

Without being bound by any theory it is believed that amino acid residues I462; G465; K489; I491; N493; and C499 as depicted figure 2 are involved in binding an epitope by an antibody comprising a variable domain as indicated herein above. Involvement in binding is preferably determined by observing a reduced binding of the variable domain to an EGFR with one or more of the amino acid residue substitutions selected from I462A; G465A; K489A; I491A; N493A; and C499A.

In one aspect, the variable domain that binds an epitope on an extracellular part of human EGFR is a variable domain that binds an epitope that is located within amino acid residues 420-480 of the sequence depicted in figure 2. Preferably the binding of the variable domain to EGFR is reduced by one or more of the following amino acid residue substitutions I462A; G465A; K489A; I491A; N493A; and C499A in EGFR. The binding of the antibody to human EGFR preferably interferes with the binding of EGF to the receptor. The epitope on EGFR is preferably a conformational epitope. In one aspect the epitope is located within amino acid residues 420-480 of the sequence depicted in figure 2, preferably within 430-480 of the sequence depicted in figure 2; preferably within 438-469 of the sequence depicted in figure 2.

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.

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 3 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 3.

- 5 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 3; or the CDR1, CDR2 and CDR3 sequences of the VH of MF3755 as depicted in Figure 3 with at most three, preferably at most two, preferably at most one amino acid substitutions.

10

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 3; or the amino acid sequence of the VH chain of MF3755 depicted in Figure 3 having at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and
15 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.

In one embodiment, the disclosure provides an antibody comprising a variable domain that binds an extracellular part of EGFR and a variable domain that binds an
20 extracellular part of LGR5,

- wherein a heavy chain variable region of said variable domain comprises at least the CDR3 sequence of an EGFR specific heavy chain variable region selected from the group consisting of MF3370; MF3755; MF4280 or MF4289 as depicted in Figure 3 or wherein a heavy chain variable region of said variable domain comprises a
25 heavy chain 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 a VH selected from the group consisting of MF3370; MF3755; MF4280 or MF4289 as depicted in Figure 3. Said variable domain preferably comprises a heavy chain variable region comprising at least the CDR3 sequence of MF3370; MF3755; MF4280 or MF4289 as
30 depicted in Figure 3.

Said variable domain preferably comprises a heavy chain variable region comprising at least the CDR1, CDR2 and CDR3 sequences of an EGFR specific heavy chain variable region selected from the group consisting of MF3370; MF3755; MF4280

- or MF4289 as depicted in Figure 3, or heavy chain variable region comprising at least CDR1, CDR2 and CDR3 sequences that differ in at most three, preferably in at most two, preferably in at most one amino acid from the CDR1, CDR2 and CDR3 sequences of an EGFR specific heavy chain variable region selected from the group consisting of
- 5 MF3370; MF3755; MF4280 or MF4289 as depicted in Figure 3. Said variable domain preferably comprises a heavy chain variable region comprising at least the CDR1, CDR2 and CDR3 sequences of MF3370; MF3755; MF4280 or MF4289 as depicted in Figure 3. A preferred heavy chain variable region is MF3755. Another preferred heavy chain variable region is MF4280.
- 10 The antibody comprising a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5, wherein the EGFR binding variable domains has a CDR3, a CDR1, CDR2, and CDR3 and/or a VH sequence as indicated herein above preferably has a variable domain that binds LGR5 that comprises at least the CDR3 sequence of an LGR5 specific heavy chain variable
- 15 region selected from the group consisting of MF5790; MF5803; MF5805; MF5808; MF5809; MF5814; MF5816; MF5817; or MF5818 as depicted in Figure 3 or a heavy chain 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 a VH selected from the group consisting of MF5790; MF5803; MF5805; MF5808; MF5809; MF5814;
- 20 MF5816; MF5817; or MF5818 as depicted in Figure 3. Said variable domain preferably comprises a heavy chain variable region comprising at least the CDR3 sequence of MF5790; MF5803; MF 5805; MF5808; MF5809; MF5814; MF5816; MF5817; or MF5818 as depicted in Figure 3.
- 25 The LGR5 variable domain preferably comprises a heavy chain variable region comprising at least the CDR1, CDR2 and CDR3 sequences of an LGR5 specific heavy chain variable region selected from the group consisting of MF5790; MF5803; MF5805; MF5808; MF5809; MF5814; MF5816; MF5817; or MF5818 as depicted in Figure 3, or heavy chain CDR1, CDR2 and CDR3 sequences that differ in at most
- 30 three, preferably in at most two, preferably in at most one amino acid from the CDR1, CDR2 and CDR3 sequences of LGR5 specific heavy chain variable region selected from the group consisting of MF5790; MF5803; MF5805; MF5808; MF5809; MF5814; MF5816; MF5817; or MF5818 as depicted in Figure 3. Said variable domain

- preferably comprises a heavy chain variable region comprising at least the CDR1, CDR2 and CDR3 sequences of MF5790; MF5803; MF5805; MF5808; MF5809; MF5814; MF5816; MF5817; or MF5818 as depicted in Figure 3. Preferred heavy chain variable regions are MF5790; MF5803; MF5814; MF5816; MF5817; or MF5818.
- 5 Particularly preferred heavy chain variable regions are MF5790; MF5814; MF5816; and MF5818; preferably MF5814, MF5818 and MF5816, heavy chain variable region MF5816 is particularly preferred. Another preferred heavy chain variable region is MF5818.
- 10 It has been shown that the antibodies comprising one or more variable domains with a heavy chain variable region MF3755 or one or more CDRs thereof have a better effectivity when used to inhibit growth of an EGFR ligand responsive cancer or cell. In the context of bispecific or multispecific antibodies, an arm of the antibody comprising a variable domain with a heavy chain variable region MF3755 or one or more CDRs
- 15 thereof combines well with an arm comprising a variable domain with a heavy chain variable region MF5818 or one or more CDRs thereof.
- VH chains of variable domains that bind EGFR or LGR5 can have one or more amino acid substitutions with respect to the sequence depicted in figure 3. A VH chain
- 20 preferably has an amino acid sequence of an EGFR or LGR5 VH of figure 3, 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 sequence of Figure 3.
- 25 CDR sequences can have one or more an amino acid residue substitutions with respect to a CDR sequence in the figures. Such one or more substitutions are for instance made for optimization purposes, preferably in order to improve binding strength or the stability of the antibody. Optimization is for instance performed by mutagenesis procedures where after the stability and/or binding affinity of the
- 30 resulting antibodies are preferably tested and an improved EGFR specific CDR sequence or LGR5 specific CDR sequence is preferably selected. A skilled person is well capable of generating antibody variants comprising at least one altered CDR sequence according to the invention. For instance, conservative amino acid

substitution may be applied. Examples of conservative amino acid substitution include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another hydrophobic residue, and the substitution of one polar residue for another polar residue, such as the substitution of arginine for lysine,
5 glutamic acid for aspartic acid, or glutamine for asparagine.

Preferably, the mentioned at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably 1, 2, 3, 4 or 5 amino acid substitutions in a VH or VL as specified herein are preferably conservative amino acid substitutions. The amino acid insertions,
10 deletions and substitutions in a VH or VL as specified herein are preferably not present in the CDR3 region. The mentioned amino acid insertions, deletions and substitutions are preferably also not present in the CDR1 and CDR2 regions. The mentioned amino acid insertions, deletions and substitutions are preferably also not present in the FR4 region.

15 The mentioned at most 15, preferably 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably 1, 2, 3, 4 or 5 amino acid substitutions are preferably conservative amino acid substitutions, the insertions, deletions, substitutions or a combination thereof are preferably not in the CDR3 region of the VH chain, preferably not in the CDR1, CDR2
20 or CDR3 region of the VH chain and preferably not in the FR4 region.

An antibody comprising a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 preferably comprises
25 - the amino acid sequence of VH chain MF3755 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF3755 as depicted in Figure 3 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 said VH; and

wherein the VH chain of the variable domain that binds LGR5 comprises
30 - the amino acid sequence of VH chain MF5790 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF5790 as depicted in Figure 3 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 said VH.

An antibody comprising a variable domain that binds an extracellular part of EGFR
5 and a variable domain that binds an extracellular part of LGR5 preferably comprises

- the amino acid sequence of VH chain MF3755 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF3755 as depicted in Figure 3 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

10 said VH; and

wherein the VH chain of the variable domain that binds LGR5 comprises

- the amino acid sequence of VH chain MF5803 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF5803 as depicted in Figure 3 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

15 amino acid insertions, deletions, substitutions or a combination thereof with respect said VH.

An antibody comprising a variable domain that binds an extracellular part of EGFR
and a variable domain that binds an extracellular part of LGR5 preferably comprises

20

- the amino acid sequence of VH chain MF3755 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF3755 as depicted in Figure 3 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 said VH; and

25

wherein the VH chain of the variable domain that binds LGR5 comprises

- the amino acid sequence of VH chain MF5814 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF5814 as depicted in Figure 3 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

30 said VH.

An antibody comprising a variable domain that binds an extracellular part of EGFR
and a variable domain that binds an extracellular part of LGR5 preferably comprises

- the amino acid sequence of VH chain MF3755 as depicted in Figure 3; or
 - the amino acid sequence of VH chain MF3755 as depicted in Figure 3 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
- 5 said VH; and
- wherein the VH chain of the variable domain that binds LGR5 comprises
- the amino acid sequence of VH chain MF5816 as depicted in Figure 3; or
 - the amino acid sequence of VH chain MF5816 as depicted in Figure 3 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
- 10 amino acid insertions, deletions, substitutions or a combination thereof with respect said VH.

- An antibody comprising a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 preferably comprises
- 15
- the amino acid sequence of VH chain MF3755 as depicted in Figure 3; or
 - the amino acid sequence of VH chain MF3755 as depicted in Figure 3 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
- said VH; and
- 20
- wherein the VH chain of the variable domain that binds LGR5 comprises
- the amino acid sequence of VH chain MF5817 as depicted in Figure 3; or
 - the amino acid sequence of VH chain MF5817 as depicted in Figure 3 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
- 25 said VH.

- An antibody comprising a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 preferably comprises
- the amino acid sequence of VH chain MF3755 as depicted in Figure 3 or
- 30
- the amino acid sequence of VH chain MF3755 as depicted in Figure 3 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
- said VH; and

wherein the VH chain of the variable domain that binds LGR5 comprises

- the amino acid sequence of VH chain MF5818 as depicted in Figure 3; or
- the amino acid sequence of VH chain MF5818 as depicted in Figure 3 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

5 amino acid insertions, deletions, substitutions or a combination thereof with respect said VH.

Additional variants of the disclosed amino acid sequences which retain EGFR or LGR5 binding can be obtained, for example, from phage display libraries which

10 contain the rearranged human IGKV1-39/IGKJ1 VL region (De Kruif et al. Biotechnol Bioeng. 2010 (106)741-50), and a collection of VH regions incorporating amino acid substitutions into the amino acid sequence of an EGFR or LGR5 VH region disclosed herein, as previously described (e.g., WO2017/069628). Phages encoding Fab regions

15 which bind EGFR or LGR5 may be selected and analyzed by flow cytometry, and sequenced to identify variants with amino acid substitutions, insertions, deletions or additions which retain antigen binding.

The light chain variable regions of the VH/VL EGFR and LGR5 variable domains of the EGFR/LGR5 antibody may be the same or different. In some embodiments, the VL

20 region of the VH/VL EGFR variable domain of the EGFR/LGR5 antibody is similar to the VL region of the VH/VL LGR5 variable domain. In certain embodiments, VL regions in the first and second VH/VL variable domains are identical.

In certain embodiments, the light chain variable region of one or both VH/VL variable

25 domains of the EGFR/LGR5 antibody comprises a common light chain variable region. In some embodiments, the common light chain variable region of one or both VH/VL variable domains comprises a germline IgVκ1-39 variable region V-segment. In certain embodiment, the light chain variable region of one or both VH/VL variable domains comprises the kappa light chain V-segment IgVκ1-39*01. IgVκ1-39 is short

30 for Immunoglobulin Variable Kappa 1-39 Gene. The gene is also known as Immunoglobulin Kappa Variable 1-39; IGKV139; IGKV1-39. External Ids for the gene are HGNC: 5740; Entrez Gene: 28930; Ensembl: ENSG00000242371. The amino acid sequence for a suitable V-region is provided in Figure 4. The V-region can be

combined with one of five J-regions. Preferred J-regions are jk1 and jk5, and the joined sequences are indicated as IGKV1-39/jk1 and IGKV1-39/jk5; alternative names are IgV κ 1-39*01/IGJ κ 1*01 or IgV κ 1-39*01/IGJ κ 5*01 (nomenclature according to the IMGT database worldwide web at imgt.org). In certain embodiments, the light chain
5 variable region of one or both VH/VL variable domains comprises the kappa light chain IgV κ 1-39*01/IGJ κ 1*01 or IgV κ 1-39*01/IGJ κ 1*05 (described in Figure 4).

In some embodiments, the light chain variable region of one or both VH/VL variable domains of the EGFR/LGR5 bispecific antibody comprises an LCDR1 comprising the
10 amino acid sequence QSISSY (described in Figure 4), an LCDR2 comprising the amino acid sequence AAS (described in Figure 4), and an LCDR3 comprising the amino acid sequence QQSYSTP (described in Figure 4) (i.e., the CDRs of IGKV1-39 according to IMGT). In some embodiments, the light chain variable region of one or both VH/VL variable domains of the EGFR/LGR5 antibody comprises an LCDR1
15 comprising the amino acid sequence QSISSY (described in Figure 4), an LCDR2 comprising the amino acid sequence AASLQS (described in Figure 4), and an LCDR3 comprising the amino acid sequence QQSYSTP (described in Figure 4).

In some embodiments, one or both VH/VL variable domains of the EGFR/LGR5
20 antibody comprise a light chain variable region comprising an amino acid sequence that is at least 90%, preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% identical or 100% identical to the amino acid sequence of set forth in Figure 4. In some embodiments, one or both VH/VL variable domains of the EGFR/LGR5 antibody comprise a light chain variable
25 region comprising an amino acid sequence that is at least 90%, preferably at least 95%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99% identical or 100% identical to the amino acid sequence of set forth in Figure 4.

For example, in some embodiments, the variable light chain of one or both VH/VL
30 variable domains of the EGFR/LGR5 antibody can have from 0 to 10, preferably from 0 to 5 amino acid insertions, deletions, substitutions, additions or a combination thereof with respect to a sequence in Figure 4. In some embodiments, the light chain variable region of one or both VH/VL variable domains of the EGFR/LGR5 antibody

comprises from 0 to 9, from 0 to 8, from 0 to 7, from 0 to 6, from 0 to 5, from 0 to 4, preferably from 0 to 3, preferably from 0 to 2, preferably from 0 to 1 and preferably 0 amino acid insertions, deletions, substitutions, additions with respect to the indicated amino acid sequence, or a combination thereof.

5

In other embodiments, the light chain variable region of one or both VH/VL variable domains of the EGFR/LGR5 antibody comprises the amino acid sequence of a sequence as depicted in Figure 4. In certain embodiments, both VH/VL variable domains of the EGFR/LGR5 antibody comprise identical VL regions. In one
10 embodiment, the VL of both VH/VL variable domains of the EGFR/LGR5 bispecific antibody comprises the amino acid sequence set forth in Figure 4. In one embodiment, the VL of both VH/VL variable domains of the EGFR/LGR5 bispecific antibody comprises the amino acid sequence set forth in Figure 4.

- 15 The EGFR/LGR5 antibody as described herein is preferably a bispecific antibody having two variable domains, one that binds EGFR and another that binds LGR5 as described herein. EGFR/LGR5 bispecific antibodies for use in the methods disclosed herein can be provided in a number of formats. Many different formats of bispecific antibodies are known in the art, and have been reviewed by Kontermann (Drug
20 Discov Today, 2015 Jul;20(7):838-47; MAbs, 2012 Mar-Apr;4(2):182-97) and in Spiess et al., (Alternative molecular formats and therapeutic applications for bispecific antibodies. Mol. Immunol. (2015) <http://dx.doi.org/10.1016/j.molimm.2015.01.003>), which are each incorporated herein by reference. For example, bispecific antibody
25 formats that are not classical antibodies with two VH/VL combinations, have at least a variable domain comprising a heavy chain variable region and a light chain variable region. This variable domain may be linked to a single chain Fv-fragment, monobody, a VH and a Fab-fragment that provides the second binding activity.

- In some embodiments, the EGFR/LGR5 bispecific antibodies used in the methods
30 provided herein are generally of the human IgG subclass (e.g., for instance IgG1, IgG2, IgG3, IgG4). In certain embodiments, the antibodies are of the human IgG1 subclass. Full length IgG antibodies are preferred because of their favorable half-life and for reasons of low immunogenicity. Accordingly, in certain embodiments, the

EGFR/LGR5 bispecific antibody is a full length IgG molecule. In an embodiment, the EGFR/LGR5 bispecific antibody is a full length IgG1 molecule.

Accordingly, in certain embodiments, the EGFR/LGR5 bispecific antibody comprises a
5 fragment crystallizable (Fc). The Fc of the EGFR/LGR5 bispecific antibody is preferably comprised of a human constant region. A constant region or Fc of the EGFR/LGR5 bispecific antibody may contain one or more, preferably not more than 10, preferably not more than 5 amino-acid differences with a constant region of a naturally occurring human antibody. For example, in certain embodiments, each Fab-
10 arm of the bispecific antibodies may further include an Fc-region comprising modifications promoting the formation of the bispecific antibody, promoting stability and/or other features described herein.

Bispecific antibodies are typically produced by cells that express nucleic acid(s)
15 encoding the antibody. Accordingly, in some embodiments, the bispecific EGFR/LGR5 antibodies disclosed herein are produced by providing a cell comprising one or more nucleic acids that encode the heavy and light chain variable regions and constant regions of the bispecific EGFR/LGR5 antibody. The cell is preferably an animal cell, more preferably a mammal cell, more preferably a primate cell, most preferably a
20 human cell. A suitable cell is any cell capable of comprising and preferably of producing the EGFR/LGR5 bispecific antibody.

Suitable cells for antibody production are known in the art and include a hybridoma cell, a Chinese hamster ovary (CHO) cell, an NS0 cell or a PER-C6 cell. Various
25 institutions and companies have developed cell lines for the large scale production of antibodies, for instance for clinical use. Non-limiting examples of such cell lines are CHO cells, NS0 cells or PER.C6 cells. In a particularly preferred embodiment said cell is a human cell. Preferably a cell is transformed by an adenovirus E1 region or a functional equivalent thereof. A preferred example of such a cell line is the PER.C6
30 cell line or equivalent thereof. In a particularly preferred embodiment said cell is a CHO cell or a variant thereof. Preferably the variant makes use of a Glutamine synthetase (GS) vector system for expression of an antibody. In one preferred embodiment, the cell is a CHO cell.

- In some embodiments, the cell expresses the different light and heavy chains that make up the EGFR/LGR5 bispecific antibody. In certain embodiments, the cell expresses two different heavy chains and at least one light chain. In one preferred
5 embodiment, the cell expresses a “common light chain” as described herein to reduce the number of different antibody species (combinations of different heavy and light chains). For example, the respective VH regions are cloned into expression vectors using methods known in the art for production of bispecific IgG (WO2013/157954; incorporated herein by reference), in conjunction with the rearranged human IGKV1
10 39/IGKJ1 (huVκ1 39) light chain, previously shown to be able to pair with more than one heavy chain thereby giving rise to antibodies with diverse specificities, which facilitates the generation of bispecific molecules (De Kruif et al. J. Mol. Biol. 2009 (387) 548 58; WO2009/157771).
- 15 An antibody producing cell that expresses a common light chain and equal amounts of the two heavy chains typically produces 50% bispecific antibody and 25% of each of the monospecific antibodies (i.e. having identical heavy light chain combinations). Several methods have been published to favor the production of the bispecific antibody over the production of the respective monospecific antibodies. Such is
20 typically achieved by modifying the constant region of the heavy chains such that they favor heterodimerization (i.e. dimerization with the heavy chain of the other heavy/light chain combination) over homodimerization. In a preferred embodiment the bispecific antibody of the invention comprises two different immunoglobulin heavy chains with compatible heterodimerization domains. Various compatible
25 heterodimerization domains have been described in the art. The compatible heterodimerization domains are preferably compatible immunoglobulin heavy chain CH3 heterodimerization domains. The art describes various ways in which such hetero-dimerization of heavy chains can be achieved.
- 30 One preferred method for producing the EGFR/LGR5 bispecific antibody is disclosed in US 9,248,181 and US 9,358,286. Specifically, preferred mutations to produce essentially only bispecific full length IgG molecules are the amino acid substitutions L351K and T366K (EU numbering) in the first CH3 domain (the ‘KK-variant’ heavy

chain) and the amino acid substitutions L351D and L368E in the second domain (the 'DE-variant' heavy chain), or vice versa. As previously described, the DE-variant and KK-variant preferentially pair to form heterodimers (so-called 'DEKK' bispecific molecules). Homodimerization of DE-variant heavy chains (DEDE homodimers) or
5 KK-variant heavy chains (KKKK homodimers) hardly occurs due to strong repulsion between the charged residues in the CH3-CH3 interface between identical heavy chains.

Accordingly, in one embodiment the heavy chain/light chain combination that
10 comprises the variable domain that binds EGFR, comprises a DE variant of the heavy chain. In this embodiment the heavy chain/light chain combination that comprises the variable domain that binds LGR5 comprises a KK variant of the heavy chain.

A candidate EGFR/LGR5 IgG bispecific antibody can be tested for binding using any
15 suitable assay. For example, binding to membrane-expressed EGFR or LGR5 on CHO cells can be assessed by flow cytometry (according to the FACS procedure as previously described in WO2017/069628). In one embodiment, the binding of a candidate EGFR/LGR5 bispecific antibody to LGR5 on CHO cells is demonstrated by flow cytometry, performed according to standard procedures known in the art.
20 Binding to the CHO cells is compared with CHO cells that have not been transfected with expression cassettes for EGFR and/or LGR5. The binding of the candidate bispecific IgG1 to EGFR is determined using CHO cells transfected with an EGFR expression construct; a LGR5 monospecific antibody and an EGFR monospecific antibody, as well as an irrelevant IgG1 isotype control mAb are included in the assay
25 as controls (e.g., an antibody which binds LGR5 and another antigen such as tetanus toxin (TT)).

The affinities of the LGR5 and EGFR Fabs of a candidate EGFR/LGR5 bispecific antibody for their targets can be measured by surface plasmon resonance (SPR)
30 technology using a BIAcore T100. Briefly, an anti-human IgG mouse monoclonal antibody (Becton and Dickinson, cat. Nr. 555784) is coupled to the surfaces of a CM5 sensor chip using free amine chemistry (NHS/EDC). Then the bsAb is captured onto the sensor surface. Subsequently, the recombinant purified antigens human EGFR

(Sino Biological Inc, cat. Nr. 11896-H07H) and human LGR5 protein are run over the sensor surface in a concentration range to measure on- and off-rates. After each cycle, the sensor surface is regenerated by a pulse of HCl and the bsAb is captured again. From the obtained sensorgrams, on- and off-rates and affinity values for binding to human LGR5 and EGFR are determined using the BIAevaluation software, as previously described for CD3 in US 2016/0368988.

An antibody as disclosed herein is typically a bispecific full length antibody, preferably of the human IgG subclass, preferably of the human IgG1 subclass. Such antibodies have good ADCC properties which can, if desired, be enhanced by techniques known in the art, have favorable half-life upon in vivo administration to humans and CH3 engineering technology exists that can provide for modified heavy chains that preferentially form heterodimers over homodimers upon co-expression in clonal cells.

ADCC activity of an antibody can be improved when the antibody itself has a low ADCC activity, by modifying the constant region of the antibody. Another way to improve ADCC activity of an antibody is by enzymatically interfering with the glycosylation pathway resulting in a reduced fucose. Several in vitro methods exist for determining the efficacy of antibodies or effector cells in eliciting ADCC. Among these are chromium-51 [Cr51] release assays, europium [Eu] release assays, and sulfur-35 [S35] release assays. Usually, a labeled target cell line expressing a certain surface-exposed antigen is incubated with antibody specific for that antigen. After washing, effector cells expressing Fc receptor CD16 are co-incubated with the antibody-labeled target cells. Target cell lysis is subsequently measured by release of intracellular label by a scintillation counter or spectrophotometry.

A bispecific antibody as disclosed herein can be ADCC enhanced. A bispecific antibody can in one embodiment be afucosylated. A bispecific antibody preferably comprises a reduced amount of fucosylation of the N-linked carbohydrate structure in the Fc region, when compared to the same antibody produced in a normal CHO cell.

The antibody that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 may further comprise one or more additional variable domains that can bind one or more further targets. The further target is preferably a protein, preferably a membrane protein
5 comprising an extracellular part. A membrane protein as used herein is a cell membrane protein, such as a protein that is in the outer membrane of a cell, the membrane that separates the cell from the outside world. The membrane protein has an extracellular part. A membrane protein is at least on a cell if it contains a transmembrane region that is in the cell membrane of the cell.

10

Antibodies with more than two variable domains are known in the art. For instance, it is possible to attach an additional variable domain to a constant part of the antibody. An antibody with three or more variable domains is preferably a multivalent multimer antibody as described in PCT/NL2019/050199 which is
15 incorporated by reference herein.

In one embodiment the antibody is a bispecific antibody comprising two variable domains, wherein one variable domain binds an extracellular part of EGFR and another variable domain binds an extracellular part of LGR5. The variable domains
20 are preferably variable domains as described herein.

A functional part of an antibody as described herein comprises at least a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5 as described herein. It thus comprises the antigen binding
25 parts of an antibody as described herein and typically contains the variable domains of the antibody. A variable domain of a functional part can be a single chain Fv-fragment or a so-called single domain antibody fragment. A single-domain antibody fragment (sdAb) is an antibody fragment with a single monomeric variable antibody domain. Like a whole antibody, it is able to bind selectively to a specific antigen. With
30 a molecular weight of only 12–15 kDa, single-domain antibody fragments are much smaller than common antibodies (150–160 kDa) which are composed of two heavy protein chains and two light chains, and even smaller than Fab fragments (~50 kDa, one light chain and half a heavy chain) and single-chain variable fragments (~25 kDa,

two variable domains, one from a light and one from a heavy chain). Single-domain antibodies by themselves are not much smaller than normal antibodies (being typically 90-100kDa). Single-domain antibody fragments are mostly engineered from heavy-chain antibodies found in camelids; these are called VHH fragments (Nanobodies®). Some fishes also have heavy-chain only antibodies (IgNAR, 'immunoglobulin new antigen receptor'), from which single-domain antibody fragments called VNAR fragments can be obtained. An alternative approach is to split the dimeric variable domains from common immunoglobulin G (IgG) from humans or mice into monomers. Although most research into single-domain antibodies is currently based on heavy chain variable domains, nanobodies derived from light chains have also been shown to bind specifically to target epitopes. Non-limiting examples of such variable domains of antibody parts are VHH, Human Domain Antibodies (dAbs) and Unibodies. Preferred antibody parts or derivatives have at least two variable domains of an antibody or equivalents thereof. Non-limiting examples of such variable domains or equivalents thereof are F(ab)-fragments and Single chain Fv fragments. A functional part of a bispecific antibody comprises the antigen binding parts of the bispecific antibody, or a derivative and/or analogue of the binding parts. As mentioned herein above, the binding part of an antibody is encompassed in the variable domain.

Also provided is an antibody or functional part, derivative and/or analogue thereof as disclosed herein (i.e., the therapeutic compound) and a pharmaceutically acceptable carrier. Such pharmaceutical compositions are useful in the treatment of cancer, in particular for the treatment of gastric, esophageal, or gastro-esophageal-junction cancer. As used herein, the term "pharmaceutically acceptable" means approved by a government regulatory agency or listed in the U.S. Pharmacopeia or another generally recognized pharmacopeia for use in animals, particularly in humans, and includes any and all solvents, salts, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil,

glycerol polyethylene glycol ricinoleate, and the like. Water or aqueous solution saline and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions. Liquid compositions for parenteral administration can be formulated for administration by injection or continuous infusion. Routes of

5 administration by injection or infusion include intravesical, intratumoral, intravenous, intraperitoneal, intramuscular, intrathecal and subcutaneous. Depending on the route of administration (e.g., intravenously, subcutaneously, intra-articularly and the like) the active compound may be coated in a material to protect the compound from the action of acids and other natural conditions that may

10 inactivate the compound.

Pharmaceutical compositions suitable for administration to human patients are typically formulated for parenteral administration, e.g., in a liquid carrier, or suitable for reconstitution into liquid solution or suspension for intravenous administration.

15 The compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Also included are solid preparations which are intended for conversion, shortly before use, to liquid preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions and emulsions.

20 The therapeutic compound disclosed can be administered according to a suitable dosage, and suitable route (e.g., intravenous, intraperitoneal, intramuscular, intrathecal or subcutaneous). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic

25 situation. In one embodiment, a subject is administered a single dose of the antibody or functional part, derivative and/or analogue thereof as disclosed herein. In some embodiments, the therapeutic compound will be administered repeatedly, over a course of treatment. For example, in certain embodiments, multiple (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) doses of the therapeutic compound are administered to a subject in

30 need of treatment. In some embodiments, administrations of the therapeutic compound may be done weekly, biweekly or monthly.

A clinician may utilize preferred dosages as warranted by the condition of the patient being treated. The dose may depend upon a number of factors, including stage of disease, etc. Determining the specific dose that should be administered based upon the presence of one or more of such factors is within the skill of the artisan. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small amounts until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired. Intermittent therapy (e.g., one week out of three weeks or three out of four weeks) may also be used.

In certain embodiments, the therapeutic compound is administered at a dose of 0.1, 0.3, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg body weight. In another embodiment, the therapeutic compound is administered at a dose of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg body weight.

In a preferred embodiment, the therapeutic compound (i.e., an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5) is provided to a subject at a dosage of 1500 mg. A flat dosage offers several advantages over body-surface or weight dosing as it reduces preparation time and reduces potential dose calculation mistakes. In some embodiments, the therapeutic compound is provided at a dosage of at least 1100 mg, preferably at a dosage of between 1100 to 2000 mg, more preferably at a dosage of between 1100 to 1800 mg. As is understood by the skilled person, the dosage can be administered over time. For example, the dosage may be administered by IV, for example with a 1-6 hour infusion, preferably a 2-4 hour infusion. In some embodiments, the therapeutic compound is administered once every 2 weeks. In some embodiments, the flat dosages disclosed herein are suitable for use in adults and/or in subjects weighing at least 35kg. Preferably, the subject is afflicted with gastric, esophageal, or gastro-esophageal- junction cancer.

In some embodiments, a premedication regimen may be used. Such a regimen may be useful to reduce the likelihood or severity of an infusion-related reaction. Generally, a steroid such as dexamethasone and/or an antihistamine such as dexchlorpheniramine, diphenhydramine, or chlorpheniramine is administered (e.g., orally, intravenously)
5 prior to antibody treatment.

The treatment method described herein is typically continued for as long as the clinician overseeing the patient's care deems the treatment method to be effective, i.e., that the patient is responding to treatment. Non-limiting parameters that indicate
10 the treatment method is effective may include one or more of the following: decrease in tumor cells; inhibition of tumor cell proliferation; tumor cell elimination; progression-free survival; appropriate response by a suitable tumor marker (if applicable).

15 With regard to the frequency of administering the therapeutic compound, one of ordinary skill in the art will be able to determine an appropriate frequency. For example, a clinician can decide to administer the therapeutic compound relatively infrequently (e.g., once every two weeks) and progressively shorten the period
20 between doses as tolerated by the patient. Exemplary lengths of time associated with the course of therapy in accordance with the claimed method include: about one week; two weeks; about three weeks; about four weeks; about five weeks; about six weeks; about seven weeks; about eight weeks; about nine weeks; about ten weeks; about eleven weeks; about twelve weeks; about thirteen weeks; about fourteen weeks; about fifteen weeks; about sixteen weeks; about seventeen weeks; about eighteen weeks;
25 about nineteen weeks; about twenty weeks; about twenty-one weeks; about twenty-two weeks; about twenty-three weeks; about twenty four weeks; about seven months; about eight months; about nine months; about ten months; about eleven months; about twelve months; about thirteen months; about fourteen months; about fifteen months; about sixteen months; about seventeen months; about eighteen months;
30 about nineteen months; about twenty months; about twenty one months; about twenty -two months; about twenty -three months; about twenty -four months; about thirty months; about three years; about four years; about five years; perpetual (e.g.,

ongoing maintenance therapy). The foregoing duration may be associated with one or multiple rounds/cycles of treatment.

The efficacy of the treatment methods provided herein can be assessed using any
5 suitable means. In one embodiment, the clinical efficacy of the treatment is analyzed using cancer cell number reduction as an objective response criterion. Patients, e.g., humans, treated according to the methods disclosed herein preferably experience improvement in at least one sign of cancer. In some embodiments, one or more of the following can occur: the number of cancer cells can be reduced; cancer recurrence is
10 prevented or delayed; one or more of the symptoms associated with cancer can be relieved to some extent. In addition, in vitro assays to determine the T cell mediated target cell lysis. In some embodiments, tumor assessment is based on CT-scan and/or MRI scans, see, e.g., the RECIST 1.1 guidelines (Response Evaluation Criteria in Solid Tumours) (Eisenhauer et al., 2009 Eur J Cancer 45:228–247). Such assessments
15 generally take place every 4-8 weeks after treatment.

In some embodiments, the tumor cells are no longer detectable following treatment as described herein. In some embodiments, a subject is in partial or full remission. In certain embodiments, a subject has an increased overall survival, median survival
20 rate, and/or progression free survival.

The therapeutic compound (i.e., an antibody or functional part, derivative and/or analogue thereof that comprises a variable domain that binds an extracellular part of EGFR and a variable domain that binds an extracellular part of LGR5) may also be
25 used in conjunction with other well-known therapies (e.g., chemotherapy or radiation therapy) that are selected for their particular usefulness against the cancer that is being treated.

Methods for the safe and effective administration of chemotherapeutic agents are
30 known to those skilled in the art. In addition, their administration is described in the standard literature. For example, the administration of many of the chemotherapeutic agents is described in the Physicians' Desk Reference (PDR), e.g., 1996 edition

(Medical Economics Company, Montvale, N.J. 07645-1742, USA); the disclosure of which is incorporated herein by reference thereto.

It will be apparent to those skilled in the art that the administration of the
5 chemotherapeutic agent(s) and/or radiation therapy can be varied depending on the
disease being treated and the known effects of the chemotherapeutic agent(s) and/or
radiation therapy on that disease. Also, in accordance with the knowledge of the
skilled clinician, the therapeutic protocols (e.g., dosage amounts and times of
administration) can be varied in view of the observed effects of the administered
10 therapeutic agents on the patient, and in view of the observed responses of the disease
to the administered therapeutic agents.

The compounds and compositions disclosed herein are useful as therapy and in
therapeutic treatments and may thus be useful as medicaments and used in a method
15 of preparing a medicament.

All documents and references, including Genbank entries, patents and published
patent applications, and websites, described herein are each expressly incorporated
herein by reference to the same extent as if were written in this document in full or in
20 part.

For the purpose of clarity and a concise description features are described herein as
part of the same or separate embodiments, however, it will be appreciated that the
scope of the invention may include embodiments having combinations of all or some of
25 the features described.

The invention is now described by reference to the following examples, which are
illustrative only, and are not intended to limit the present invention. While the
invention has been described in detail and with reference to specific embodiments
30 thereof, it will be apparent to one of skill in the art that various changes and
modifications can be made thereto without departing from the spirit and scope
thereof.

EXAMPLES

As used herein “MFXXXX” wherein X is independently a numeral 0-9, refers to a Fab comprising a variable domain wherein the VH has the amino acid sequence identified by the 4 digits depicted in figure 3. Unless otherwise indicated the light chain variable region of the variable domain typically has a sequence of figure 4b. The light chain in the examples has a sequence as depicted in figure 4a. “MFXXXX VH” refers to the amino acid sequence of the VH identified by the 4 digits. The MF further comprises a constant region of a light chain and a constant region of a heavy chain that normally interacts with a constant region of a light chain. The VH/variable region of the heavy chains differs and typically also the CH3 region, wherein one of the heavy chains has a KK mutation of its CH3 domain and the other has the complementing DE mutation of its CH3 domain (see for reference PCT/NL2013/050294 (published as WO2013/157954) and figure 5d and 5e. Bispecific antibodies in the examples have an Fc tail with a KK/DE CH3 heterodimerization domain, a CH2 domain and a CH1 domain as indicated in figure 5, a common light chain as indicated in figure 4a and a VH as specified by the MF number. For example a bispecific antibody indicated by MF3755 xMF5816 has the above general sequences and a variable domain with a VH with the sequence of MF3755 and a variable domain with a VH with the sequence of MF5816.

The amino acid and nucleic acid sequences of the various heavy chain variable regions (VH) are indicated in Figure 3. Bispecific antibodies EGFR/LGR5, MF3755xMF5816; comprising heavy chain variable regions MF3755 and MF5816 and a common light chain and including modifications for enhanced ADCC from afucosylation, among other LGR5 and EGFR combinations as depicted in Figure 3 have been shown to be effective in WO2017/069628.

Generation of bispecific antibodies

Bispecific antibodies were generated by transient co-transfection of two plasmids encoding IgG with different VH domains, using a proprietary CH3 engineering technology to ensure efficient heterodimerisation and formation of bispecific antibodies. The common light chain is also co-transfected in the same cell, either on the same plasmid or on another plasmid. In our applications (e.g. WO2013/157954

and WO2013/157953; incorporated herein by reference) we have disclosed methods and means for producing bispecific antibodies from a single cell, whereby means are provided that favor the formation of bispecific antibodies over the formation of monospecific antibodies. These methods can also be favorably employed in the present invention. Specifically, preferred mutations to produce essentially only bispecific full length IgG molecules are amino acid substitutions at positions 351 and 366, e.g. L351K and T366K (numbering according to EU numbering) in the first CH3 domain (the 'KK-variant' heavy chain) and amino acid substitutions at positions 351 and 368, e.g. L351D and L368E in the second CH3 domain (the 'DE-variant' heavy chain), or vice versa (see figure 5d and 5e). It was previously demonstrated in the mentioned applications that the negatively charged DE-variant heavy chain and positively charged KK-variant heavy chain preferentially pair to form heterodimers (so-called 'DEKK' bispecific molecules). Homodimerization of DE-variant heavy chains (DE-DE homodimers) or KK-variant heavy chains (KK-KK homodimers) hardly occurs due to strong repulsion between the charged residues in the CH3-CH3 interface between identical heavy chains.

VH genes of variable domain that bind LGR5 described above were cloned into the vector encoding the positively charged CH3 domain. The VH genes of variable domain that bind EGFR such as those disclosed in WO 2015/130172 (incorporated herein by reference) were cloned into vector encoding the negatively charged CH3 domain. Suspension growth-adapted 293F Freestyle cells were cultivated in T125 flasks on a shaker plateau until a density of 3.0×10^6 cells/ml. Cells were seeded at a density of $0.3\text{--}0.5 \times 10^6$ viable cells/ml in each well of a 24-deep well plate. The cells were transiently transfected with a mix of two plasmids encoding different antibodies, cloned into the proprietary vector system. Seven days after transfection, the cellular supernatant was harvested and filtered through a 0.22 μm filter (Sartorius). The sterile supernatant was stored at 4°C until purification of the antibodies.

30 IgG purification and quantification

Purifications were performed under sterile conditions in filter plates using Protein-A affinity chromatography. First, the pH of the medium was adjusted to pH 8.0 and subsequently, IgG-containing supernatants were incubated with protein A Sepharose

CL-4B beads (50% v/v) (Pierce) for 2hrs at 25°C on a shaking platform at 600 rpm. Next, the beads were harvested by filtration. Beads were washed twice with PBS pH 7.4. Bound IgG was then eluted at pH 3.0 with 0.1 M citrate buffer and the eluate was immediately neutralized using Tris pH 8.0. Buffer exchange was performed by
5 centrifugation using multiscreen Ultracel 10 multiplates (Millipore). The samples were finally harvested in PBS pH7.4. The IgG concentration was measured using Octet. Protein samples were stored at 4°C.

To determine the amount of IgG purified, the concentration of antibody was
10 determined by means of Octet analysis using protein-A biosensors (Forte-Bio, according to the supplier's recommendations) using total human IgG (Sigma Aldrich, cat. nr. I4506) as standard.

The following bispecific antibodies are suitable for use in this example and for
15 use in the methods of the invention: MF3370xMF5790, MF3370x5803, MF3370x5805, MF3370x5808, MF3370x5809, MF3370x5814, MF3370x5816, MF3370x5817, MF3370x5818, MF3755xMF5790, MF3755x5803, MF3755x5805, MF3755x5808, MF3755x5809, MF3755x5814, MF3755x5816, MF3755x5817, MF3755x5818, MF4280xMF5790, MF4280x5803, MF4280x5805, MF4280x5808,
20 MF4280x5809, MF4280x5814, MF4280x5816, MF4280x5817, MF4280x5818, MF4289xMF5790, MF4289x5803, MF4289x5805, MF4289x5808, MF4289x5809, MF4289x5814, MF4289x5816, MF4289x5817, and MF4289x5818. Each bispecific antibody comprises two VH as specified by the MF numbers capable of binding EGFR and LGR5 respectively, further comprises an Fc tail with a KK/DE CH3
25 heterodimerization domain as indicated by SEQ ID NO: 136 (Figure 5d) and SEQ ID NO: 138 (Figure 5e), respectively, a CH2 domain as indicated by SEQ ID NO: 134 (Figure 5c) and a CH1 domain as indicated by SEQ ID NO:131 (Figure 5a), a common light chain as indicated by SEQ ID NO: 121 (Figure 4).

30 **Example 1: Evaluation of anti-EGFRxanti-LGR5 candidate against various cancers:**

Mice model selection

Crown Biosciences Inc. has developed a collection of patient-derived xenograft (PDX) models derived from surgically resected human primary tumors (Crown Bioscience database, <http://hubase.crownbio.com>). PDX models are clinically and molecularly annotated and faithfully represent the clinical epidemiology of the respective tumors. These models can be injected subcutaneously in the flanks of immunodeficient mice. Different cancer models were tested for the expression of EGFR and LGR5 as analyzed by RNA sequencing (RNAseq) (see **TABLE 1**). A set of esophageal and gastric cancer PDX models, found to exhibit high EGFR and LGR5 expression levels, were selected to test the efficacy of an MF3755 x MF5816 bispecific antibody. For esophageal cancer 4 PDX models were chosen and for gastric cancer 8 PDX models were chosen. The detailed information of PDX models, including cancer subtype, known driver mutations, EGFR/LGR5 expression and time to randomization are described in (**TABLE 1**).

TABLE 1
Characteristics of PDX models originating from esophageal and gastric cancer patients.

LGR5 and EGFR expression was determined by RNA sequencing (RNAseq). Mutations status of oncogenic drivers was determined by genomic analysis. Time to randomization (when the mean tumor reaches approximately 100-150 mm³), along with growth characteristics of the tumors are also mentioned. ESCC = Esophageal squamous cell carcinoma; EAC = Esophageal adenocarcinoma; ADC = Adenocarcinoma; N/A = Not available or not applicable.

Model	Sub-type	Known mutations	EGFR expression	LGR5 expression	Time to randomization	Growth Characteristics
Cancer type: Esophageal Cancer						
ES0042	ESCC	TP53 - R196T	6.3346	3.6394	~14-24days	Ulceration
ES0199	ESCC	TP53 - R342T; MLH1 - V384D	7.0919	2.5491	~18-40days	
ES0201	ESCC	PIK3CA - E545K	5.7606	1.8863	~18-46days	
ES2356	ESCC	TP53 - R248Q; PIK3CA- H1047R; CDKN2A-W110T; UGT1A1 - G71R; UGT1A8- G71R	7.0124	3.2865	~62days	
ES11065	ESCC	ATM – W57T	4.5	4.2		
Cancer type: Gastric Cancer						
GA0429	ADC		4.3486	4.4366	~22 days	Cachexia

GA2434	ADC	BRAF - V600E; PTEN - R130Ter	3.4811	5.1645	~30 days	
GA6212	ADC	KRAS - G12C; UGT1A1 - G71R; UGT1A8- G71R	4.9772	3.3944	~29 days	Ulceration
GA6822	ADC	UGT1A1 - G71R; UGT1A8- G71R	3.9165	3.9266	~22 days	
GA6833	ADC	PIK3CA - E545K; UGT1A1 - G71R; UGT1A8- G71R	4.1009	5.4768	~25-45 days	
GA6864	N/A		9.2291	3.5282	~25-45 days	Ulceration
GA6891	ADC	UGT1A1 - G71R; UGT1A8- G71R	3.8613	6.1634	~30 days	
GA3236	ADC	UGT1A1 - G71R; UGT1A8- G71R	4.047	6.027	~10 - 14 days	

Methods

Fresh tumor tissue for the inoculation was harvested from mice bearing established primary human tumors. Tumor fragments (2-3 mm in diameter) were inoculated subcutaneously at the upper right dorsal flank of 6-8 week old female BALB/c Nude or NOD/SCID mice depending on the model. When tumors reached 100-150 mm³ in size mice were randomized. A total of 6 mice per model were enrolled, 3 control mice and 3 MF3755 xMF5816 bispecific antibody treated mice. For 6 weeks, mice received 0.5mg bispecific antibody/week intraperitoneally, in 200 µl injection volume (approx. 25 mg/kg/week) regardless of their weight. Control mice received PBS (200 µl). After 6 weeks of treatment period tumor growth was monitored for additional 3 weeks. If the mice reached humane endpoint they were sacrificed earlier than 63 days.

Results:

From the 8 gastric PDX models tested, MF3755 xMF5816 bispecific antibody treatment dramatically reduced tumor growth in 6 models (Figure 6a). From these 8 models, 3 models (GA0429, GA6833 and GA6891) showed lower tumor volume at the end of the observation period than at the beginning of the treatment suggesting a strong tumor inhibition of the bispecific antibody in gastric cancer. Model GA2434 gave certain response. As regarding the esophageal PDX models, all four tested models responded to bispecific antibody treatment, with the strongest response of model ES2356 (Figure 6b). Results showing statistically significant ($p < 0.0001$) efficacy of treatment with the MF3755 xMF5816 bispecific antibody of this example were obtained with model ES11065.

Example 2: Dose expansion, and efficacy of anti-EGFRxanti-LGR5 antibody for patients having EAC, GAC and GEJAC:**Phase 1 dose escalation study in advanced solid tumors****Study Design**

A phase 1 open-label multicenter study was performed with an initial dose escalation part to determine the recommended phase 2 dose (RP2D) of an anti-EGFRxanti-LGR5 bispecific antibody for solid tumors in mCRC patients with a starting dose of 5 mg flat dose. Once the RP2D is established, the antibody is further evaluated in an expansion part of the study, including in patients diagnosed with EAC, GAC and GEJAC.

Safety, PK, immunogenicity and preliminary antitumor activity of the antibody is characterized in all patients, and biomarker analyses, including EGFR and LGR5 status is performed.

Dose Escalation

In the dose escalation part, patients with metastatic colorectal cancer (mCRC) adenocarcinoma previously treated in the metastatic setting with standard approved therapy including oxaliplatin, irinotecan and a fluoropyrimidine (5-FU and/or capecitabine), with or without an anti-angiogenic, and an anti-EGFR for KRAS and NRAS wild-type RASwt are treated.

A PK model was generated based on the available bispecific antibody serum concentration data from the preliminary and GLP cynomolgus monkey toxicology studies. Following allometric scaling, this model was used to predict antibody exposure in humans. The antibody starting dose is 5 mg (flat dose) IV, every 2 weeks, with 4-week cycles. Up to 11 dose levels will be investigated: 5, 20, 50, 90, 150, 225, 335, 500, 750, 1100 and 1500 mg (flat dose). The administered dose, dose increments, and frequency of dosing for each patient and each cohort is subject to change based on patient safety, PK and PD data, however the dose will not exceed 4500 mg per cycle.

Dose-limiting toxicity (DLT)

Any of the following clinical toxicities and/or laboratory abnormalities occurring during the first cycle (28 days) and considered by the investigator to be related to antibody treatment will be considered DLT:

- Hematologic toxicities:
 - Grade 4 neutropenia (absolute neutrophil count [ANC] $<0.5 \times 10^9$ cells/L) for ≥ 7 days
 - Grade 3-4 febrile neutropenia
 - Grade 4 thrombocytopenia
 - Grade 3 thrombocytopenia associated with bleeding episodes
 - Other grade 4 hematologic toxicity
- Grade 3-4 non-hematologic AEs and laboratory toxicities with the exception of:
 - Grade 3-4 infusion-related reactions

- Grade 3 skin toxicity that recovers to grade ≤ 2 within 2 weeks with optimal treatment
- Grade 3 diarrhea, nausea and/or vomiting that recover to grade ≤ 1 or baseline within 3 days with optimal treatment
- Grade 3 electrolyte abnormalities that resolve with optimal treatment within 48 hours
- Grade 3-4 liver abnormalities lasting ≤ 48 hours
- Any liver function abnormalities that meet the definition of Hy's law.
- Any drug-related toxicity lasting ≥ 15 days that prevents the next two administrations.

Dose expansion

In the expansion part, the bispecific antibody will be administered at the RP2D in patients having EAC, GAC or GEJAC. Once the RP2D has been defined, additional patients will be treated with this dose and schedule to further characterize safety, tolerability, PK and immunogenicity of antibody, and to perform a preliminary assessment of antitumor activity and biomarker evaluations. The malignancies treated will be known to co-express both targets (i.e. LGR5 and EGFR) and may have prior indication of sensitivity to EGFR inhibition.

Antibody treatment in patients with EAC, GAC or GEJAC will be explored for example, 10 to 20 patients for each indication with potential expansion up to 40 patients, conditional on signs of preliminary anti-tumor activity). The safety of the RP2D will be continuously evaluated during the expansion part of the study by the Safety Monitoring Committee. If the incidence of DLTs exceeds the predefined threshold of 33% for any cohort, enrolment will be paused for this cohort and a full review of the safety, PK, and biomarkers will be performed by the SMC in order to determine if it is safe to continue accrual in that cohort. The overall safety of the drug will also be interrogated at that time.

Investigational therapy and regimen

The anti-EGFRxanti-LGR5 bispecific antibody is formulated as a clear liquid solution for IV infusion. IV infusion is performed every 2 weeks using standard infusion

procedures, with a starting dose of 5 mg (flat dose), and with a recommended phase 2 dose of 1500 mg (flat dose). Dose escalation was halted once the RP2D had been reached. Infusions must be administered over a minimum of 4 hours during Cycle 1. Subsequent infusions after Cycle 1 can be reduced to 2 hours at the investigator's discretion and in the absence of IRRs.

A cycle is considered 4 weeks. For each patient, a 6-hour observation period was implemented following infusion start for the initial antibody infusion, a 4-hour period for the second infusion, and a minimum of 2 hours for all subsequent administrations, corresponding to at least the duration of the infusion. Antibody was administered as a 2 to 4-hour IV infusion every 2 weeks, with 4-week cycles. Day 1 of the subsequent cycle was on Day 29 or after recovery from any adverse effects associated with the previous cycle.

Treatment duration

Study treatment is administered until confirmed progressive disease (as per RECIST 1.1), unacceptable toxicity, withdrawal of consent, patient non-compliance, investigator decision (e.g. clinical deterioration), or antibody interruption >6 consecutive weeks. Patients are followed up for safety for at least 30 days following the last antibody infusion and until recovery or stabilization of all related toxicities, and for disease progression and survival status for 12 months.

Efficacy assessments

Tumor assessment is based on CT/MRI with contrast per RECIST 1.1 (Eisenhauer et al., 2009 Eur J Cancer 45:228–247), every 8 weeks after treatment start. Objective responses must be confirmed at least 4 weeks after first observation. Bone scans are performed as clinically indicated for patients with bone metastases at baseline or suspected lesions on study. Circulating blood tumor markers, including carcinoembryonic antigen (CEA), are evaluated at screening and on Day 1 of each cycle.