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(54) **Title:** HGF-MET AGONIST FOR USE IN THE TREATMENT OF CANCER AND COLORECTAL FIBROSIS

(57) **Abstract:** The present invention relates to treatment of cancer using agonist anti-MET antibodies or fragments thereof. In particular, the invention relates to treatment of colorectal cancer using agonist anti-MET antibodies or fragments, typically colorectal cancer associated with chronic inflammation and/or gene mutations in the colon and in the gastrointestinal tract in general. The invention further relates to treating intestinal fibrosis using agonist anti-MET antibodies.

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HGF-MET AGONIST FOR USE IN THE TREATMENT OF CANCER AND COLORECTAL FIBROSIS

FIELD OF THE INVENTION

The present invention relates to treatment of cancer using agonist anti-MET antibodies or fragments thereof. In particular, the invention relates to treatment of colorectal cancer using agonist anti-MET antibodies or fragments, typically colorectal cancer associated with chronic inflammation and/or gene mutations in the colon and in the gastrointestinal tract in general. The invention further relates to treating intestinal fibrosis using agonist anti-MET antibodies.

BACKGROUND

HGF is a pleiotropic cytokine of mesenchymal origin that mediates a characteristic array of biological functions including cell proliferation, motility, differentiation and survival. The HGF receptor, also known as MET, is expressed by a variety of tissues including all epithelia, the endothelium, muscle cells, neuronal cells, osteoblasts, hematopoietic cells and various components of the immune system. HGF and MET signalling plays an essential role during embryo development, where it guides migration of precursor cells and determines cell survival or death.

In adults, HGF/MET signalling is ordinarily quiescent and is resumed during wound healing and tissue regeneration. Some cancers and tumours hijack HGF/MET signalling in order to promote the survival and proliferation of the tumour in the host organism.

Therefore, inhibiting the HGF-MET axis has become a popular target for anti-cancer treatment. Use of an agonist of HGF-MET as an anti-cancer therapy has not previously been demonstrated.

Colorectal cancer is the third most common cancer in terms of incidence, with a 5 year survival rate of approximately 65%. Subjects particularly at risk include those with inflammatory bowel disease, or genetic predispositions (e.g. those with a history of hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome), Gardner syndrome, or familial adenomatous polyposis (FAP)). Treatment with surgery (e.g. colectomy) and/or chemotherapy and radiotherapy can be effective, but lead to significant loss in standard of living. There is therefore a need for effective therapies for colorectal cancer.

SUMMARY OF THE INVENTION

HGF is a known pro-oncogenic factor that plays a key role in tumorigenesis of various tissues and organs, including the gastro-intestinal tract (Gherardi *et at.* Nat Rev Cancer

12:89-103, 2012; Vermeulen et al. Nat Cell Biol. 12:468-476, 2010; Samame Perez-Vargas et al. Int J Mol Sci. 14:1 8056-1 8077, 2013; Stein et al. Nat Med. 15: 59-67, 2009, each of which is incorporated herein by reference). As a result, *inhibiting* HGF-MET has become a popular target for anti-cancer treatment. However, it is surprisingly identified herein that an *agonist* of HGF-MET acts as an effective anti-cancer therapy.

Therefore, in a first aspect, the invention provides a method of treating cancer in a subject, the method comprising administering to the subject an HGF-MET agonist. In certain preferred embodiments, the HGF-MET agonist is an anti-MET agonist antibody or antigen fragment thereof.

Surprisingly, an HGF-MET agonist (e.g. an anti-MET agonist antibody) is particularly effective as a therapy for colorectal cancer, as demonstrated herein. Without wishing to be bound by theory, it is hypothesised that stimulation of the HGF-MET signalling promotes regeneration and homeostatic mechanisms of intestinal epithelial cells, thereby dampening potentially oncogenic mechanisms (Nakamura et al., *J Gastroenterol Hepatol.* 1:1 88-202, 201 1, incorporated herein by reference).

In a further aspect, the invention provides a method of treating colorectal fibrosis in a subject, the method comprising administering to a subject an HGF-MET agonist.

Advantageously, it is also demonstrated herein that HGF-MET agonists are surprisingly effective at treating colorectal cancer in inflamed guts. Further advantageously, it is also demonstrated herein that HGF-MET agonists are surprisingly effective at treating colorectal fibrosis in inflamed guts.

This is particularly advantageous since patients suffering from colorectal inflammation are at increased risk of colorectal cancer and also from fibrosis. For instance, patients suffering from inflammatory bowel disease (IBD; i.e. Crohn's Disease or ulcerative colitis) are predisposed to colorectal cancer and will advantageously benefit from the methods described herein. Furthermore, HGF-MET agonists can also relieve the symptoms of the underlying IBD itself. Therefore the methods of the invention will lead to a synergistic effect in IBD patients whereby colorectal cancer in these patients can be treated and, furthermore, the patient's IBD is also treated. Similarly, the methods of the invention will lead to a synergistic effect in IBD patients whereby colorectal fibrosis in these patients can be treated and, furthermore, the patient's IBD is also treated.

Therefore, in a further preferred embodiment, the method of the invention is a method of treating colorectal cancer in a patient or subject identified as at increased risk of colorectal cancer. In certain embodiments, the subject has been diagnosed with colorectal inflammation prior to administration of the HGF-MET agonist. In certain embodiments of the methods

described herein, the subject has IBD (ulcerative colitis or Crohn's Disease). In preferred such embodiments, the HGF-MET agonist administered to the subject is an anti-MET agonist antibody.

Similarly, in a further preferred embodiment, the method of the invention is a method of treating colorectal fibrosis in a patient or subject identified as at increased risk of colorectal fibrosis. In certain embodiments, the subject has been diagnosed with colorectal inflammation prior to administration of the HGF-MET agonist. In certain embodiments of the methods described herein, the subject has IBD (ulcerative colitis or Crohn's Disease). In preferred such embodiments, the HGF-MET agonist administered to the subject is an anti-MET agonist antibody.

In a further aspect is provided an HGF-MET agonist for use in methods of treatment of cancer (e.g. colorectal cancer) in a subject as described herein. In preferred such embodiments, the HGF-MET agonist is an anti-MET agonist antibody or antigen fragment thereof.

In a further aspect is provided an HGF-MET agonist for use in methods of treatment of colorectal fibrosis in a subject as described herein. In preferred such embodiments, the HGF-MET agonist is an anti-MET agonist antibody or antigen fragment thereof.

In a further aspect is provided a pharmaceutical composition for use in methods of treating cancer (e.g. colorectal cancer) as described herein, wherein the pharmaceutical composition comprises an HGF-MET agonist and a pharmaceutically acceptable excipient or carrier. In preferred such embodiments, the HGF-MET agonist is an anti-MET agonist antibody or antigen fragment thereof.

In a further aspect is provided a pharmaceutical composition for use in methods of treating colorectal fibrosis as described herein, wherein the pharmaceutical composition comprises an HGF-MET agonist and a pharmaceutically acceptable excipient or carrier. In preferred such embodiments, the HGF-MET agonist is an anti-MET agonist antibody or antigen fragment thereof.

In a preferred embodiment of all aspects of the invention, the subject or patient is a human.

In a preferred embodiment of all aspects of the invention, the HGF-MET agonist is a full agonist.

In a preferred embodiment of all aspects of the invention, the HGF-MET agonist is an anti-MET agonist antibody.

DRAWINGS

Figure 1. AOM/DSS colon carcinogenesis model: body weight and disease activity index (DAI) over time. Colitis-associated colorectal cancer was induced in BALB/c mice by i.p. administration of azoxymethane (AOM) at a dose of 12,5 mg/kg followed by three cycles of dextran sodium sulphate (DSS) in the drinking water at a concentration of 6%. Each cycle consisted of 7 days of DSS treatment followed by 14 days of regular water. Starting from day 1, mice were randomized into 4 arms which received treatment with: (i) vehicle only (PBS); (ii) the MET agonistic 71D6 antibody at a dose of 1 mg/kg ; (iii) the MET agonistic 71D6 antibody at a dose of 5 mg/kg; (iv) the MET antagonistic antibody 74C8-OA at a dose of 5 mg/kg. An additional, fifth control arm contained 7 mice that received no AOM-DSS or antibody and served as healthy control. Antibodies were delivered by i.p. injection two times a week. During the whole course of the experiment, mouse weight was monitored on a regular basis, and the clinical symptoms of ulcerative colitis were assessed by determining fecal blood, rectal bleeding and stool consistency. Each parameter was given a score from 0 (absence of the symptom) to 3 (maximal manifestation of the symptom). Scores relative to the single parameters were summed together to give rise to the DAI ranging from 0 to 9. (A) Body weight over time. (B) DAI over time.

Figure 2. AOM/DSS colon carcinogenesis model: colon length and specific weight. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend. At autopsy, colon specimens were collected and washed through. Tissues were weighed and their length was determined using a ruler. (A) Colon length. (B) Colon specific weight.

Figure 3. AOM/DSS colon carcinogenesis model: representative images of intestinal tumours. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend. At autopsy, colon specimens were collected and washed through. Following length and weight measurement, colons were opened with a longitudinal cut and stained with 1% Alcian Blue solution to highlight tumour borders. Colon specimens were analyzed by placing the flattened tissue under a stereo-microscope with their inner (lumen) side towards the lens, and photographed. Magnification: 1X.

Figure 4. AOM/DSS colon carcinogenesis model: analysis of mean tumour number, mean tumour volume and total tumour burden. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend, and carcinogenesis was quantified using a stereo-microscope. (A) Tumour number. The number of polips in each colon sample was scored directly. (B) Mean tumour volume. Tumour photographs were analyzed using Image J software (National Instututes of Health) and the volume of the polips was calculated

using the formula $V = \frac{3}{4}\pi(X/2)\cdot(Y/2)^2$, where V is the volume of the polip, and X and Y are the major and minor dimensions of the polip section, respectively (in mm). (C) Total tumour burden. Total tumour burden (volume) was calculated by multiplying the mean tumour volume by the number of tumours in each mouse.

Figure 5. AOM/DSS colon carcinogenesis model: histological analysis of colon samples. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend. Following tissue processing and paraffin embedding, colon specimens were cut using a microtome and prepared for histological and immunohistochemical analysis. Here, we show representative images of colon sections stained with hematoxylin and eosin. Magnification: 100X.

Figure 6. AOM/DSS colon carcinogenesis model: immunohistochemical analysis of collagen deposition. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend. Colon sections were analyzed by immunohistochemistry. Here, we show representative images of colon sections stained with picro-sirius red, which highlights collagen deposition, a hallmark of tissue fibrosis. Magnification: 400X.

Figure 7. AOM/DSS colon carcinogenesis model: immunohistochemical analysis of myofibroblast content. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend. Colon sections were analyzed by immunohistochemistry. Here, we show representative images of colon sections stained with anti-alpha smooth muscle actin (α-SMA) antibodies, which specifically identify myofibroblasts. Myofibroblast accumulation is a hallmark of fibrosis. Magnification: 400X.

Figure 8. AOM/DSS colon carcinogenesis model: immunohistochemical analysis of TGF-β expression. Colitis-associated cancer was induced in BALB/c mice as described in Figure 1 legend. Colon sections were analyzed by immunohistochemistry. Here, we show representative images of colon sections stained with anti-transforming growth factor beta (TGF-β) antibodies. TGF-β signalling has been demonstrated to be frequently deregulated in human cancers, including colorectal cancer. While in normal or premalignant cells it usually acts as a tumour suppressor, in advanced cancer it is frequently overexpressed and the growth inhibitory function switch to an oncogenic one thus promoting tumour cell proliferation and invasion.

Figure 9. AOM colon carcinogenesis model: colon length and specific weight. Mutagenesis-induced colorectal cancer was induced in BALB/c mice by i.p. administration of azoxymethane (AOM) at a dose of 5 mg/kg once a week for 6 weeks. Starting from day 1, mice were randomized into 2 arms of 21 mice each which received treatment with 71D6 (at a dose of 5 mg/kg) or vehicle only (PBS). Antibody was administered two times a week by i.p.

injection. An additional, third control arm contained 7 mice that received no AOM or antibody and served as healthy control. Mice were sacrificed 8 weeks after the last AOM injection, i.e. 14 weeks after the experiment started. At autopsy, colons were collected and washed through. Explanted colons were measured using a ruler and weighed. (A) Colon length. (B) Colon specific weight.

Figure 10. AOM colon carcinogenesis model: tumour incidence and number. Mutagenesis-induced colorectal cancer was induced in BALB/c mice as described in Figure 9 legend. Following measurements, colons were cut open longitudinally to expose tumour masses. Tissues were stained ex vivo with a 1% Alcian Blue solution in order to highlight tumour borders. Polips were counted using a stereo-microscope. (A) Tumour incidence. (B) Mean tumour number.

Figure 11. AOM colon carcinogenesis model: representative images of colon tumours. Representative images of the tumours quantified in Figure 10. Arrows indicate macroscopically evident tumour masses. Magnification: 1X.

Figure 12. Intestinal (colorectal) inflammation model. Dextran sodium sulphate (DSS) was added to the drinking water of Balb/c mice for 10 days. On day 10, DSS treatment was interrupted and mice were put back on normal water. Starting from day 1, mice were randomized into 7 arms which received treatment with 71G3, 71D6, 71G2 (at a dose of 1 mg/kg or 5 mg/kg) or vehicle only (PBS). An additional, eighth control arm received no DSS or antibody and served as healthy control. Mice were sacrificed on day 12, i.e. 2 days after DSS administration was interrupted. At autopsy, colons were collected, washed through, and their length was determined using a ruler. Following measurement, colons were embedded in paraffin and processed for histological analysis. During the whole course of the experiment, mouse weight was monitored on a regular basis, and the clinical symptoms of ulcerative colitis were assessed by determining faecal blood, rectal bleeding and stool consistency. Each parameter was given a score from 0 (absence of the symptom) to 3 (maximal manifestation of the symptom). Scores relative to the single parameters were summed together to give rise to the DAI ranging from 0 to 9. (A) Body weight over time (% relative to time 0). (B) DAI over time. (C) Colon length at autopsy. Data of the 1 mg/kg arms and of the 5 mg/kg arms are shown in separate graphs for clarity.

Figure 13. Intestinal (colorectal) inflammation model. BALB/c mice were exposed to dextran sodium sulphate (DSS) as described in Figure 12 legend. At autopsy, colons were collected, measured, and then embedded in paraffin and processed for histological analysis. Colon sections were stained with hematoxylin and eosin, examined by microscopy, and photographed. Experimental arm, antibody dose and magnification are indicated close to

each image.

DETAILED DESCRIPTION

As used herein, the terms "MET protein" or "MET antigen" or "MET" are used interchangeably and refer to the receptor tyrosine kinase that, in its wild-type form, binds Hepatocyte Growth Factor (HGF). "MET" as used herein refers to human MET unless otherwise specified. The terms "human MET protein" or "human MET receptor" or "human MET" or "hMET" are used interchangeably to refer to human MET (GenBank accession number: X54559), including the native human MET protein naturally expressed in the human host and/or on the surface of human cultured cell lines, as well as recombinant forms and fragments thereof and also naturally occurring mutant forms. The terms "mouse MET protein" or "mouse MET receptor" or "mouse MET" or "mMET" are used interchangeably to refer to mouse MET (GenBank accession number: NM_008591), including the native mouse MET protein naturally expressed in the mouse host and/or on the surface of mouse cultured cell lines, as well as recombinant forms and fragments thereof and also naturally occurring mutant forms.

As used herein, "HGF-MET agonist" and "MET agonist" are used interchangeably to refer to non-native agents that promote signalling via the MET protein - i.e. agents other than HGF that bind MET and increase MET signalling. Agonist activity on binding of MET by MET agonists is indicated by molecular and/or cellular responses that (at least partially) mimic the molecular and cellular responses induced upon HGF-MET binding. Suitable methods for measuring MET agonist activity are described herein, including the Examples. A "full agonist" is a MET agonist that increases MET signalling in response to binding to an extent at least similar, and optionally exceeding, the extent to which MET signalling increases in response to binding of the native HGF ligand. Examples of the level of MET signalling induced by "full agonists", as measured by different methods of determining MET signalling, are provided herein.

HGF-MET agonists may be small molecules, binding proteins such as antibodies or antigen binding fragments, aptamers or fusion proteins. A particular example of a MET agonist is an anti-MET agonist antibody.

As used herein, "treatment" or "treating" refers to effective therapy of the relevant condition (cancer (e.g. colorectal cancer) or fibrosis) - that is, an improvement in the health of the subject. Treatment may be therapeutic or prophylactic treatment - that is, therapeutic treatment of subjects suffering from the condition, or prophylactic treatment of a subject so as to reduce their risk of contracting the condition or the severity of the condition once

contracted. Therapeutic treatment may be characterised by improvement in the health of the subject compared to prior to treatment. Therapeutic treatment may be characterised by improvement in the health of the subject compared to a comparable control subject that has not received treatment. Prophylactic treatment may be characterised by improvement in the
5   health of the subject compared to a control subject (or population of control subjects) that has not been treated. Prophylactic treatment may also be characterised by prevention of the deterioration of the health of the subject.

As used herein, the term "antibody" includes an immunoglobulin having a combination of two heavy and two light chains which have significant specific immuno-
10   reactive activity to an antigen of interest (e.g. human MET). The terms "anti-MET antibodies" or "MET antibodies" are used interchangeably herein to refer to antibodies which exhibit immunological specificity for human MET protein. "Specificity" for human MET does not exclude cross-reaction with species homologues of MET. In particular, "agomAbs" as used herein refers MET antibodies that bind to both human MET and mouse MET.

15   "Antibody" as used herein encompasses antibodies of any human class (e.g. IgG, IgM, IgA, IgD, IgE) as well as subclasses/isotypes thereof (e.g. IgG1, IgG2, IgG3, IgG4, IgA1). Antibody as used herein also refers to modified antibodies. Modified antibodies include synthetic forms of antibodies which are altered such that they are not naturally occurring, e.g., antibodies that comprise at least two heavy chain portions but not two
20   complete heavy chains (such as, domain deleted antibodies or minibodies); multispecific forms of antibodies (e.g., bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen); heavy chain molecules joined to scFv molecules and the like. In addition, the term "modified antibody" includes multivalent forms of antibodies (e.g., trivalent, tetravalent, etc., antibodies that bind to three or more copies of the
25   same antigen).

Antibodies described herein may possess antibody effector function, for example one or more of antibody dependent cell-mediated cytotoxicity (ADCC), complement dependent cytotoxicity (CDC) and antibody dependent cellular phagocytosis (ADCP). Alternatively, in certain embodiments antibodies for use according to the invention have an Fc region that
30   has been modified such that one or more effector functions, for example all effector functions, are abrogated.

Antibodies comprise light and heavy chains, with or without an interchain covalent linkage between them. An antigen-binding fragment of an antibody includes peptide fragments that exhibit specific immuno-reactive activity to the same antigen as the antibody
35   (e.g. MET). Examples of antigen-binding fragments include scFv fragments, Fab fragments,

and F(ab')2 fragments.

As used herein, the terms "variable region" and "variable domain" are used interchangeably and are intended to have equivalent meaning. The term "variable" refers to the fact that certain portions of the variable domains VH and VL differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its target antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called "hypervariable loops" in each of the VL domain and the VH domain which form part of the antigen binding site. The first, second and third hypervariable loops of the VLambda light chain domain are referred to herein as L1(A), L2(A) and L3(A) and may be defined as comprising residues 24-33 (L1 (A), consisting of 9, 10 or 11 amino acid residues), 49-53 (L2(A), consisting of 3 residues) and 90-96 (L3(A), consisting of 5 residues) in the VL domain (Morea et al., Methods 20, 267-279, 2000). The first, second and third hypervariable loops of the VKappa light chain domain are referred to herein as L1(K), L2(K) and L3(K) and may be defined as comprising residues 25-33 (L1 (K), consisting of 6, 7, 8, 11, 12 or 13 residues), 49-53 (L2(K), consisting of 3 residues) and 90-97 (L3(K), consisting of 6 residues) in the VL domain (Morea et al., Methods 20, 267-279, 2000). The first, second and third hypervariable loops of the VH domain are referred to herein as H1, H2 and H3 and may be defined as comprising residues 25-33 (H1 , consisting of 7, 8 or 9 residues), 52-56 (H2, consisting of 3 or 4 residues) and 91-105 (H3, highly variable in length) in the VH domain (Morea et al., Methods 20, 267-279, 2000).

Unless otherwise indicated, the terms L1, L2 and L3 respectively refer to the first, second and third hypervariable loops of a VL domain, and encompass hypervariable loops obtained from both Vkappa and Vlambda isotypes. The terms H1, H2 and H3 respectively refer to the first, second and third hypervariable loops of the VH domain, and encompass hypervariable loops obtained from any of the known heavy chain isotypes, including γ, ε, δ, α or μ.

The hypervariable loops L1, L2, L3, H1, H2 and H3 may each comprise part of a "complementarity determining region" or "CDR", as defined below. The terms "hypervariable loop" and "complementarity determining region" are not strictly synonymous, since the hypervariable loops (HVs) are defined on the basis of structure, whereas complementarity determining regions (CDRs) are defined based on sequence variability (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991) and the limits of the HVs and the CDRs may be different in some VH and VL domains.

The CDRs of the VL and VH domains can typically be defined as comprising the following amino acids: residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain, and residues 31-35 or 31-35b (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain; (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991). Thus, the HVs may be comprised within the corresponding CDRs and references herein to the "hypervariable loops" of VH and VL domains should be interpreted as also encompassing the corresponding CDRs, and vice versa, unless otherwise indicated.

The more highly conserved portions of variable domains are called the framework region (FR), as defined below. The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β-sheet configuration, connected by the three hypervariable loops. The hypervariable loops in each chain are held together in close proximity by the FRs and, with the hypervariable loops from the other chain, contribute to the formation of the antigen-binding site of antibodies. Structural analysis of antibodies revealed the relationship between the sequence and the shape of the binding site formed by the complementarity determining regions (Chothia et al., J. Mol. Biol. 227, 799-817, 1992; Tramontane et al., J. Mol. Biol, 215, 175-182, 1990). Despite their high sequence variability, five of the six loops adopt just a small repertoire of main-chain conformations, called "canonical structures". These conformations are first of all determined by the length of the loops and secondly by the presence of key residues at certain positions in the loops and in the framework regions that determine the conformation through their packing, hydrogen bonding or the ability to assume unusual main-chain conformations.

As used herein, the term "CDR" or "complementarity determining region" means the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat et al., J. Biol. Chem. 252, 6609-6616, 1977, by Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991, by Chothia et al., J. Mol. Biol. 196, 901-917, 1987, and by MacCallum et al., J. Mol. Biol. 262, 732-745, 1996, where the definitions include overlapping or subsets of amino acid residues when compared against each other. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth for comparison. Preferably, the term "CDR" is a CDR as defined by Kabat based on sequence comparisons.

Table 1: CDR definitions.

	CDR Definitions		
	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1]Residue numbering follows the nomenclature of Kabat et al., supra

[2]Residue numbering follows the nomenclature of Chothia et al., supra

[3]Residue numbering follows the nomenclature of MacCallum et al., supra

As used herein, the term "framework region" or "FR region" includes the amino acid residues that are part of the variable region, but are not part of the CDRs (e.g., using the Kabat definition of CDRs). Therefore, a variable region framework is between about 100-120 amino acids in length but includes only those amino acids outside of the CDRs. For the specific example of a heavy chain variable domain and for the CDRs as defined by Kabat et al., framework region 1 corresponds to the domain of the variable region encompassing amino acids 1-30; framework region 2 corresponds to the domain of the variable region encompassing amino acids 36-49; framework region 3 corresponds to the domain of the variable region encompassing amino acids 66-94, and framework region 4 corresponds to the domain of the variable region from amino acids 103 to the end of the variable region. The framework regions for the light chain are similarly separated by each of the light claim variable region CDRs. Similarly, using the definition of CDRs by Chothia et al. or McCallum et al. the framework region boundaries are separated by the respective CDR termini as described above. In preferred embodiments the CDRs are as defined by Kabat.

In naturally occurring antibodies, the six CDRs present on each monomeric antibody are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding site as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the heavy and light variable domains show less inter-molecular variability in amino acid sequence and are termed the framework regions. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, these framework regions act to form a scaffold that provides for positioning the six CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding site formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This

complementary surface promotes the non-covalent binding of the antibody to the immunoreactive antigen epitope. The position of CDRs can be readily identified by one of ordinary skill in the art.

As used herein, the term "hinge region" includes the portion of a heavy chain
5   molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux et al., J. Immunol. 161, 4083-4090, 1998). MET antibodies comprising a "fully human" hinge region may contain one of the hinge region
10   sequences shown in Table 2 below.

Table 2: Human hinge sequences.

IgG	Upper hinge	Middle hinge	Lower hinge
IgG1	EPKSCDKTHT (SEQ ID NO:199)	CPPCP (SEQ ID NO:200)	APELLGGP (SEQ ID NO:201)
IgG3	ELKTPLGDTTHT (SEQ ID NO:202)	CPRCP (EPKSCDTPPPCPRCP)$_3$ (SEQ ID NO:203)	APELLGGP (SEQ ID NO:204)
IgG4	ESKYGPP (SEQ ID NO:205)	CPSCP (SEQ ID NO:206)	APEFLGGP (SEQ ID NO:207)
IgG42	ERK (SEQ ID NO:208)	CCVECPPPCP (SEQ ID NO:209)	APPVAGP (SEQ ID NO:210)

As used herein the term "CH2 domain" includes the portion of a heavy chain molecule
15   that extends, e.g., from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked
20   branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

As used herein, the term "fragment" refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or
25   antibody chain. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (i.e., with the

intact antibody from which they were derived) for antigen binding (i.e., specific binding to MET). As used herein, the term "fragment" of an antibody molecule includes antigen-binding fragments of antibodies, for example, an antibody light chain variable domain (VL), an antibody heavy chain variable domain (VH), a single chain antibody (scFv), a F(ab')2 fragment, a Fab fragment, an Fd fragment, an Fv fragment, and a single domain antibody fragment (DAb). Fragments can be obtained, e.g., via chemical or enzymatic treatment of an intact or complete antibody or antibody chain or by recombinant means.

As used herein, "subject" and "patient" are used interchangeably to refer to a human individual.

Method of treating cancer

Effective treatment of cancer with an HGF-MET agonist has not been previously demonstrated.

It is surprisingly demonstrated herein that administration of an HGF-MET agonist (i.e. an agonist of MET that is not HGF) effectively treats cancer in two models. In particular, the MET agonist treated colorectal cancer in a mutagenesis model, where mice with genetic mutations were treated such that the tumour incidence and number of tumours was reduced compared to untreated controls, and also, further advantageously, compared to administration of the native MET ligand (HGF). Furthermore, administration of a MET agonist also prevented development of tumours in a model of intestinal (colorectal) inflammation-induced tumour formation. Notably, a MET *antagonist* agent failed to treat cancer in either model.

Accordingly, in a first aspect there is provided a method of treating cancer comprising administering to a subject in need thereof an HGF-MET agonist. Also provided is an HGF-Met agonist (e.g. a MET agonist antibody) for use in treating cancer, or for the manufacture of a medicament for treating cancer.

Cancers particularly suitable to be treated according to the methods described herein include cancers of epithelial origin. Cancers particularly suitable to be treated according to the claimed methods are gastrointestinal cancers, for example: oesophageal cancer, stomach cancer, pancreatic cancer, liver cancer, gallbladder cancer, colorectal cancer and anal cancer.

Cancers associated with chronic inflammation are also particularly suited to being treated according to the provided methods. For example, liver cancer is associated with inflammation caused by hepatitis virus infection, stomach cancer is associated with

inflammation caused by *Helicobacter pylori* infection, and colorectal cancer is associated with intestinal inflammation. Accordingly, in certain embodiments, the method is a method of treating a cancer associated with chronic inflammation. In certain embodiments, the method is a method of treating liver cancer. In certain embodiments, the method is a method of treating stomach cancer.

As demonstrated herein, HGF-MET agonists are particularly effective at treating colorectal cancer. Therefore, in a preferred embodiment of the methods described herein, the method is a method of treating colorectal cancer. Also provided is an HGF-Met agonist (e.g. a MET agonist antibody) for use in treating colorectal cancer, or for the manufacture of a medicament for treating colorectal cancer.

Treatment of cancer, such as colorectal cancer, can be therapeutic or prophylactic treatment - that is, therapeutic treatment of subjects suffering from the condition, or prophylactic treatment of a subject so as to reduce their risk of contracting the condition or the severity of the condition once contracted. Therefore, in certain embodiments, treatment of cancer (such as colorectal cancer) is therapeutic. In certain embodiments, therapeutic treatment can be characterised by a decrease in the number of tumours or cancerous polyps in a subject that has been administered a MET agonist compared to before administration of the MET agonist. In certain embodiments, treatment of cancer (such as colorectal cancer) can be characterised by a decrease in the size or volume of tumours or cancerous polyps compared to before administration of the MET agonist. In certain embodiments, treatment can be characterised by decrease in the number, size and/or volume of tumours or cancerous polyps in the subject compared to a control subject that has not been administered a MET agonist.

In further embodiments of methods for treatment of colorectal cancer, therapeutic treatment may be further characterised by a decrease in the extent of colon fibrosis in the subject compared to before administration of the MET agonist. Means for determining the extent of fibrosis would be familiar to the skilled person and include, for example, determining the extent of collagen deposition in a representative biopsy.

In certain embodiments, treatment of cancer, for example colorectal cancer, may be prophylactic treatment. In certain embodiments, prophylactic treatment may be characterized by a decrease in the number of tumours or cancerous polyps in a subject (or population of subjects) that has been administered a Met agonist compared to a control subject (or population of control subjects) that has not been administered a MET agonist. In certain embodiments, prophylactic treatment of cancer, for example colorectal cancer, can be characterized by a decrease in the size or volume of tumours or cancerous polyps in a

subject (or population of subjects) that has been administered a Met agonist compared to a control subject (or population of control subjects) that has not received a MET agonist.

In further embodiments, prophylactic treatment of colorectal cancer can be further characterised by a decrease in the extent of colon fibrosis in a subject (or population of subjects) that has been administered a MET agonist compared to a control subject (or population of control subjects) that has not been administered a MET agonist. Means for determining the extent of fibrosis would be familiar to the skilled person and include, for example, determining the extent of collagen deposition in a representative biopsy.

As will be appreciated by the skilled person, a "control subject" as used herein refers to a subject of comparable disease state to the subject being administered the HGF-MET agonist.

Method of treating colorectal fibrosis

It is further surprisingly demonstrated herein that administration of an HGF-MET agonist (i.e. an agonist of MET that is not HGF) effectively treats colorectal fibrosis. A subject is particularly susceptible to colorectal fibrosis when suffering from intestinal inflammation.

Accordingly, in a further aspect is provided a method of treating colorectal fibrosis, comprising administering to a subject an HGF-MET agonist. Means for determining the extent of fibrosis in a subject would be familiar to the skilled person and include, for example, determining the extent of collagen deposition in a representative biopsy. The use of an HGF-MET agonist for use for treating colorectal fibrosis, or for the manufacture of a medicament for treating colorectal fibrosis, is also provided.

In certain embodiments of methods for treatment of colorectal fibrosis, treatment may be therapeutic treatment. In certain embodiments, therapeutic treatment can be characterised by a decrease in the extent of colon fibrosis in the subject compared to before administration of the MET agonist. In certain embodiments, therapeutic treatment can be characterised by a decrease in the extent of colon fibrosis in the subject compared to a control subject that has not been administered a MET agonist.

In certain embodiments, treatment of colorectal cancer can be prophylactic treatment. In certain embodiments, prophylactic treatment may be characterised by a decrease in the extent of colon fibrosis in a subject (or population of subjects) that has been administered a MET agonist compared to a control subject (or population of control subjects) that has not been administered a MET agonist.

As will be appreciated by the skilled person, a "control subject" as used herein refers to a subject of comparable disease state to the subject being administered the HGF-MET

agonist.

Subject or Patient

As surprisingly demonstrated herein, administration of an HGF-MET agonist effectively treats cancer in a subject. It is further demonstrated that HGF-MET agonists are particularly effective at treating colorectal cancer, especially in patients predisposed or at risk of developing colorectal cancer.

Patients at increased risk of colorectal cancer are those who are more likely to develop colorectal cancer compared to an otherwise comparable healthy individual. Factors known to increase risk for colorectal cancer include, for example, age of >65 years, male gender, smoking, obesity, increased alcohol intake, increased red or processed meat intake. Methods described herein for treating colorectal cancer will be particularly effective at treating subjects having one or more of these risk factors.

Accordingly, in certain embodiments is provided a method of treating colorectal cancer comprising administering to a subject in need thereof an HGF-MET agonist, wherein the subject has been identified as at increased risk of colorectal cancer. In certain such embodiments, the subject has one or more risk factors selected from the group consisting of: age of >65 years, male gender, smoking, obesity, increased alcohol intake, increased red or processed meat intake.

Certain genetic conditions are also known risk factors for developing colorectal cancer. For example, hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome), Gardner syndrome, and familial adenomatous polyposis (FAP) are syndromes known to increase risk of a subject developing colorectal cancer. Accordingly, in certain embodiments, the subject has a predisposition to developing colorectal cancer. In certain such embodiments, the subject has FAP, HNPCC or Gardner syndrome.

A significant risk factor for colorectal cancer and also for colorectal fibrosis is intestinal inflammation, in particular colorectal inflammation. Identifying subjects with colorectal inflammation would be within the ability of the skilled person. For example, colorectal inflammation can be identified visually via endoscope, histologically via biopsy, or by measuring a marker of colorectal inflammation such as faecal calprotectin. Colorectal inflammation is characteristic of patients suffering from inflammatory bowel disease, for example Crohn's Disease or ulcerative colitis.

As demonstrated in the Examples, methods described herein are particularly effective in subjects with colorectal inflammation. In a model of gut inflammation, a HGF-MET agonist effectively reduced both tumour burden and colorectal fibrosis.

Accordingly, in embodiments of all aspects of the claimed methods, the subject has been diagnosed with colorectal inflammation prior to administration of the HGF-MET agonist. In certain embodiments, the subject has inflammatory bowel disease (IBD), for example Crohn's Disease or ulcerative colitis.

Administration

It will be appreciated that, as used herein, administration of an HGF-MET agonist (for example an anti-MET agonist antibody) to a subject refers to administration of an effective amount of the agonist.

In certain embodiments, the HGF-MET agonist is administered at a dose in the range of from about 0.1 mg/kg to about 10 mg/kg per dose. In certain embodiments, the HGF-MET agonist is administered at a dose in the range of from 0.5 mg/kg to about 10 mg/kg. That is, a dose of about 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg. In certain preferred embodiments, the HGF-MET agonist is administered at a dose in the range of from about 1 mg/kg to about 5mg/kg. In certain preferred embodiments, the HGF-MET agonist is administered at a dose of 1 mg/kg or 5 mg/kg.

Suitable routes for administration of the HGF-MET agonist (for example an anti-MET agonist antibody) to a subject would be familiar to the skilled person. Preferably the MET agonist is administered parenterally. In certain preferred embodiments, the HGF-MET agonist is administered per os (p.o.), subcutaneously (s.c.), intravenously (i.v.), intradermally (i.d.), intramuscularly (i.m.) or intraperitoneally (i.p.). In certain preferred embodiments, the HGF-MET agonist is a MET agonist antibody and is administered intravenously.

The HGF-MET agonist (for example anti-MET agonist antibody) can be administered according to a regimen that maintains an effective level of the agonist in the subject. The skilled person is familiar with suitable dosage regimens. For example, in certain embodiments, the HGF-MET agonist (e.g. MET agonist antibody) is administered according to a dosage regimen of at least once per week - that is, a dose is administered approximately every 7 days or more frequently. In certain embodiments, the HGF-MET agonist (e.g. MET agonist antibody) is administered 1-3 times a week (i.e. 1, 2 or 3 times a week). In certain preferred embodiments, the HGF-MET agonist (e.g. MET agonist antibody) is administered twice per week. In certain preferred embodiments, the HGF-MET agonist is a MET agonist antibody and is administered once per week or twice per week.

For the methods described herein, the HGF-MET agonist (e.g. MET agonist antibody) is administered for a period sufficient to achieve effective treatment. The skilled person is able to determine the necessary treatment period for any individual patient. In certain

embodiments, the HGF-MET agonist (e.g. a MET agonist antibody) is administered for a treatment period of at least 1 week. In certain embodiments, the HGF-MET agonist (e.g. a MET agonist antibody) is administered for a treatment period of at least 2 weeks, at least 3 weeks, or at least 4 weeks. In certain embodiments, the HGF-MET agonist (e.g. a MET agonist antibody) is administered for a treatment period of at least 1 month, at least 2 months or at least 3 months. In certain preferred embodiments, the HGF-MET agonist is a MET agonist antibody and is administered for a treatment period of 3 months.

It will be appreciated that the HGF-MET agonist (e.g. a MET agonist antibody) may be administered according to any combination of the described doses, dosage regimens and treatment periods. For example, in certain embodiments, the HGF-MET agonist (e.g. a MET agonist antibody) may be administered according to a dosage regimen of twice per week, at a dose of from 1mg/kg to 5 mg/kg, for a period of at least 3 months. Other embodiments of the methods explicitly include other combinations of the recited doses, dosage regimens and treatment periods.

HGF-MET agonist

It is demonstrated in the Examples below that an HGF-Met agonist effectively treats cancer, in particular colorectal cancer. It is also demonstrated that an HGF-Met agonist effectively treats colorectal fibrosis. Therefore, in all aspects of the invention, an HGF-MET agonist is to be administered to a subject or patient to treat the indicated condition (i.e. cancer (e.g. colorectal cancer) or colorectal fibrosis). "HGF-MET agonist" and "MET agonist" are used interchangeably to refer to non-native agents that promote signalling via the MET protein - i.e. agents other than HGF that bind MET and increase MET signalling. Such agents may be small molecules, binding proteins such as antibodies or antigen binding fragments, aptamers or fusion proteins. A particular example of a MET agonist is an anti-MET agonist antibody.

Agonist activity on binding of MET by the MET agonists described herein is indicated by molecular and/or cellular responses that (at least partially) mimic the molecular and cellular responses induced upon HGF-MET binding.

Methods for determining MET agonism according to the invention, for example by MET agonist antibodies and antigen binding fragments, would be familiar to the skilled person. For example, MET agonism may be indicated by molecular responses such as phosphorylation of the MET receptor and/or cellular responses, for example those detectable in a cell scattering assay, an anti-apoptosis assay and/or a branching morphogenesis assay.

MET agonism may be determined by the level of phosphorylation of the MET receptor

upon binding. In this context, a MET agonist antibody or antigen binding fragment, for example, causes auto-phosphorylation of MET in the absence of receptor-ligand binding - that is, binding of the antibody or antigen binding fragment to MET results in phosphorylation of MET in the absence of HGF. Phosphorylation of MET may be determined by assays

5　　known in the art, for example Western Blotting or phospho-MET ELISA (as described in Basilico *etal., J Clin Invest.* 124, 3172-31 86, 2014, incorporated herein by reference).

MET agonism may alternatively be measured by induction of HGF-like cellular responses. MET agonism can be measured using assays such as a cell scattering assay, an anti-apoptosis assay and/or a branching morphogenesis assay. In this context, a MET

10　　agonist, for example an antibody or antigen binding fragment, induces a response in cellular assays such as these that resembles (at least partially) the response observed following exposure to HGF.

For example, a MET agonist (for example a MET agonist antibody) may increase cell scattering in response to the antibody compared to cells exposed to a control antibody (e.g.

15　　IgG1).

By way of further example, a MET agonist (for example a MET agonist antibody) may exhibit a protective potency against drug-induced apoptosis with an EC_{50} of less than 32 nM. By way of further example, a MET agonist (for example a MET agonist antibody) may exhibit an E_{max} cellular viability of greater than 20% compared to untreated cells.

20　　By way of further example, a MET agonist (for example a MET agonist antibody) may increase the number of branches per spheroid in cell spheroid preparations exposed to the antibody or antigen binding fragment.

It is preferred that the MET agonists used according to the invention promote MET signalling to a magnitude of at least 70% of the natural ligand, HGF - that is, that the

25　　agonists are "full agonists". In certain embodiments, the MET agonists promote signalling to a magnitude of at least 80%, optionally at least 85%, at least 90%, at least 95% or at least 96%, at least 97%, at least 98%, at least 99% or at least 100% of HGF.

In certain embodiments, if MET agonism is determined using a phosphorylation assay, the MET agonist, e.g. a MET antibody, exhibits a potency for MET with an EC_{50} of

30　　< 1nM. In certain embodiments, the MET agonist, e.g. a MET antibody, exhibits a potency for MET agonism of an E_{MAX} of at least 80% (as a percentage of maximal HGF-induced activation).

In certain embodiments, if MET agonism is measured in a cell scattering assay, the MET agonist, for example a MET antibody or antigen binding fragment, induces an increase

35　　in cell scattering at least equivalent to 0.1 nM homologous HGF when the antibody

concentration is 0.1-1 nM.

In certain embodiments, if MET agonism is measured in an anti-apoptosis assay, the MET agonist (for example a MET antibody or fragment thereof) exhibits an EC_{50} no more than 1.1x that of HGF. In certain embodiments, if MET agonism is measured in an anti-apoptosis assay, the MET agonist (for example a MET antibody or fragment thereof) exhibits an E_{max} cellular viability of greater than 90% that observed for HGF.

In certain embodiments, if MET agonism is measured in a branching morphogenesis assay, cells treated with the MET agonist (e.g. a MET antibody or antigen binding fragment) exhibit greater than 90% of the number of branches per spheroid induced by the same (non-zero) concentration of HGF.

HGF-MET agonists particularly preferred in all aspects of the invention are anti-MET agonist antibodies, also referred to herein as "MET agonist antibodies", "agonist antibodies" and grammatical variations thereof. In other words, MET agonist antibodies (or antigen binding fragments thereof) for use according to the invention bind MET and promote cellular signalling via MET.

As demonstrated in the Examples, 71D6 is a MET agonist antibody that effectively treats cancer (in particular colorectal cancer) and also colorectal fibrosis. 71D6 binds an epitope on the SEMA domain of MET, in particular an epitope on blade 4-5 of the SEMA β-propeller. MET agonists binding an epitope on the SEMA domain of MET, in particular blade 4-5 of the SEMA β-propeller have therefore been demonstrated to lead to effective treatment of (colorectal) cancer. Antibody 71G2 has similar effects to 71D6 and also binds the SEMA domain of MET, in particular blade 4-5 of the SEMA β-propeller.

Thus, in certain embodiments is provided a method of treating cancer (for example colorectal cancer), or a method of treating colorectal fibrosis, comprising administering a MET agonist antibody or antigen binding fragment thereof, wherein the antibody or antigen binding fragment binds an epitope in the SEMA domain of MET. In certain preferred embodiments, the antibodies or fragments thereof binds an epitope located on a blade of the SEMA β-propeller. In certain embodiments, the epitope is located on blade 4 or 5 of SEMA β-propeller. In certain preferred embodiments, the antibody or antigen binding fragment binds an epitope located between amino acids 314-372 of MET.

As shown in the Examples, MET agonist antibodies binding the SEMA domain of MET, including 71D6 and 71G2, have been shown to bind to an epitope on MET that includes residue Ile367 and residue Asp371. Mutation at either of these residues impairs binding of the antibodies to MET, with mutation of both residues completely abrogating binding.

Therefore, in certain preferred embodiments is provided a method of treating cancer (e.g. colorectal cancer), or a method of treating colorectal fibrosis, comprising administering a MET agonist antibody or antigen binding fragment thereof, wherein the antibody or antigen binding fragment recognises an epitope comprising the amino acid residue Ile367. In certain preferred embodiments is provided a method of treating cancer (e.g. colorectal cancer), or a method of treating colorectal fibrosis, comprising administering a MET agonist antibody or antigen binding fragment thereof, wherein the antibody or antigen binding fragment recognises an epitope comprising the amino acid residueAsp371 .

In certain preferred embodiments, the antibody or antigen binding fragment binds an epitope comprising the amino acid residues Ile367 and Asp372 of MET.

As well as MET agonist antibodies binding the SEMA domain, also described herein are agonist antibodies binding other MET domains. For example, 71G3 binds an epitope on the PSI domain of MET. As demonstrated in the Examples, antibody 71G3 exhibits similar potency to 71D6 for reducing intestinal inflammation. 71G3 will therefore also be effective at treating colorectal cancer in a manner similar to 71D6. Similarly, 71G3 will also be effective at treating colorectal fibrosis in a manner similar to 71D6.

Thus, in certain embodiments is provided a method of treating cancer (for example colorectal cancer), or a method of treating colorectal fibrosis, comprising administering a MET agonist antibody or antigen binding fragment thereof, wherein the antibody or antigen binding fragment binds an epitope in the PSI domain of MET. In certain preferred embodiments, the antibody or antigen binding fragment binds an epitope located between amino acids 546 and 562 of MET.

As shown in the Examples, MET agonist antibodies binding the PSI domain of MET, including 71G3, have been shown to bind to an epitope on MET that includes residue Thr555. Mutation at this residue completely abrogated binding of the PSI-binding agonist antibodies to MET.

Therefore, in certain preferred embodiments is provided a method of treating cancer (e.g. colorectal cancer), or a method of treating colorectal fibrosis, comprising administering a MET agonist antibody or antigen binding fragment thereof, wherein the antibody or antigen binding fragment recognises an epitope comprising the amino acid residue Thr555.

Examples of MET agonist antibodies particularly suitable for use in treating cancer (for example colorectal cancer), or for use in treating colorectal fibrosis, are those having a combination of CDRs corresponding to the CDRs of an anti-MET antibody described herein. Therefore, in certain embodiments, the antibody or antigen binding fragment comprises a combination of VH and VL CDR sequences corresponding to a combination of VH CDRs

from a MET agonist antibody described in Table 3 and the corresponding combination of VL CDRs for the same antibody in Table 4.

In certain such embodiments, the antibody or antigen binding fragment comprises a combination of CDRs corresponding to a combination of VH CDRs from a MET agonist antibody described in Table 3 and the corresponding combination of VL CDRs for the same antibody in Table 4, and further having VH and VL domains with at least 90%, optionally at least 95%, optionally at least 99%, preferably 100% sequence identity with the corresponding VH and VL sequences of the antibody described in Table 6. By way of clarification, in such embodiments the permitted variation in percentage identity of the VH and VL domain sequences is not in the CDR regions.

As demonstrated in the Examples, 71D6 is a MET agonist antibody that is a "full agonist" of MET. That is, on binding of 71D6 to MET, the signalling response is similar to or even exceeds the response to binding of the native HGF ligand. 71 D6 is demonstrated to effectively treat (colorectal) cancer. Therefore in certain preferred embodiments is provided a method of treating cancer (e.g. colorectal cancer) comprising administering an HGF-MET agonist that is a full agonist - that is, an agonist that upon binding promotes MET signalling to an extent of at least 70% of MET signalling upon HGF binding. Examples for measuring MET agonism and examples of the effects of full agonists have already been described herein.

Examples of MET full agonists, such as anti-MET antibodies that are full agonists include 71D6 and 71G2, as demonstrated in the Examples. Therefore in particularly preferred embodiments is provided a method of treating cancer (e.g. colorectal cancer), or a method of treating colorectal fibrosis, comprising administering a MET agonist antibody or antigen binding fragment thereof that is a full agonist of MET. In preferred such embodiments, the antibody or fragment comprises a combination of CDRs having the corresponding CDR sequences of antibody 71D6 (SEQ ID Nos: 30, 32, 34, 107, 109, and 111), of antibody 71G2 (SEQ ID NOs: 44, 46, 48, 121, 123, and 125), or of antibody 71G3 (SEQ ID Nos: 9, 11, 13, 86, 88, and 90).

In preferred embodiments of all aspects, the MET agonist is a MET agonist antibody or antigen binding fragment thereof having HCDR1 of [71 D6] SEQ ID NO: 30, HCDR2 of SEQ ID NO: 32, HCDR3 of SEQ ID NO: 34, LCDR1 of SEQ ID NO: 107, LCDR2 of SEQ ID NO: 109, and LCDR3 of SEQ ID NO: 111. In preferred such embodiments, the antibody or antigen binding fragment comprises: a VH domain comprising SEQ ID NO: 163 or a sequence at least 90% identical thereto, optionally at least 95%, at least 98% or at least 99% identical thereto; and a VL domain comprising SEQ ID NO: 164 or a sequence at least 95%

thereto optionally at least 98% or at least 99% identical thereto. By way of clarification, in such embodiments the permitted variation in percentage identity of the VH and VL domain sequences is not in the CDR regions.

MET agonist antibodies for use as described herein can take various different embodiments in which both a VH domain and a VL domain are present. The term "antibody" herein is used in the broadest sense and encompasses, but is not limited to, monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), so long as they exhibit the appropriate immunological specificity for a human MET protein and for a mouse MET protein. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes) on the antigen, each monoclonal antibody is directed against a single determinant or epitope on the antigen.

"Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable domain thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, bi-specific Fab's, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, a single chain variable fragment (scFv) and multispecific antibodies formed from antibody fragments (see Holliger and Hudson, Nature Biotechnol. 23:1 126-1136, 2005, the contents of which are incorporated herein by reference).

In preferred embodiments of all aspects provided herein, the MET agonist antibody or antigen-binding fragment thereof is bivalent.

In non-limiting embodiments, the MET antibodies provided herein may comprise CH1 domains and/or CL domains, the amino acid sequence of which is fully or substantially human. Therefore, one or more or any combination of the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may be fully or substantially human with respect to its amino acid sequence. Such antibodies may be of any human isotype, for example IgG1 or IgG4.

Advantageously, the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may all have fully or substantially human amino acid sequence. In the context of the constant region of a humanised or chimeric antibody, or an antibody fragment, the term "substantially human" refers to an amino acid sequence identity

of at least 90%, or at least 92%, or at least 95%, or at least 97%, or at least 99% with a human constant region. The term "human amino acid sequence" in this context refers to an amino acid sequence which is encoded by a human immunoglobulin gene, which includes germline, rearranged and somatically mutated genes. Such antibodies may be of any human isotype, with human IgG4 and IgG1 being particularly preferred.

MET agonist antibodies may also comprise constant domains of "human" sequence which have been altered, by one or more amino acid additions, deletions or substitutions with respect to the human sequence, excepting those embodiments where the presence of a "fully human" hinge region is expressly required. The presence of a "fully human" hinge region in the MET antibodies of the invention may be beneficial both to minimise immunogenicity and to optimise stability of the antibody.

The MET agonist antibodies may be of any isotype, for example IgA, IgD, IgE IgG, or IgM. In preferred embodiments, the antibodies are of the IgG type, for example IgG1, IgG2a and b, IgG3 or IgG4. IgG1 and IgG4 are particularly preferred. Within each of these sub-classes it is permitted to make one or more amino acid substitutions, insertions or deletions within the Fc portion, or to make other structural modifications, for example to enhance or reduce Fc-dependent functionalities.

In non-limiting embodiments, it is contemplated that one or more amino acid substitutions, insertions or deletions may be made within the constant region of the heavy and/or the light chain, particularly within the Fc region. Amino acid substitutions may result in replacement of the substituted amino acid with a different naturally occurring amino acid, or with a non-natural or modified amino acid. Other structural modifications are also permitted, such as for example changes in glycosylation pattern (e.g. by addition or deletion of N- or O-linked glycosylation sites). Depending on the intended use of the MET antibody, it may be desirable to modify the antibody of the invention with respect to its binding properties to Fc receptors, for example to modulate effector function.

In certain embodiments, the MET antibodies may comprise an Fc region of a given antibody isotype, for example human IgG1, which is modified in order to reduce or substantially eliminate one or more antibody effector functions naturally associated with that antibody isotype. In non-limiting embodiments, the MET antibody may be substantially devoid of any antibody effector functions. In this context, "antibody effector functions" include one or more or all of antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and antibody-dependent cellular phagocytosis (ADCP).

The amino acid sequence of the Fc portion of the MET antibody may contain one or more mutations, such as amino acid substitutions, deletions or insertions, which have the

effect of reducing one or more antibody effector functions (in comparison to a wild type counterpart antibody not having said mutation). Several such mutations are known in the art of antibody engineering. Non-limiting examples, suitable for inclusion in the MET antibodies described herein, include the following mutations in the Fc domain of human IgG4 or human IgG1 : N297A, N297Q, LALA (L234A, L235A), AAA (L234A, L235A, G237A) or D265A (amino acid residues numbering according to the EU numbering system in human IgG1).

In certain embodiments of all aspects of the invention, therefore, the anti-MET agonist antibody is an agonist antibody of both human MET and mouse MET.

Pharmaceutical compositions

Also provided in accordance with the invention are pharmaceutical compositions for use in the methods described herein. Therefore in a further aspect of the invention is provided a pharmaceutical composition comprising an HGF-MET agonist, for example an anti-MET agonist antibody, and a pharmaceutically acceptable excipient or carrier for use in a method according to the invention. Suitable pharmaceutically acceptable carriers and excipients would be familiar to the skilled person. Examples of pharmaceutically acceptable carriers and excipients suitable for inclusion in pharmaceutical compositions of the invention include sodium citrate, glycine, polysorbate (e.g. polysorbate 80) and saline solution.

In certain embodiments, the MET agonist, for example anti-MET agonist antibody, is administered to the subject parenterally, preferably intravenously (i.v.). In certain embodiments the MET agonist, for example anti-MET agonist antibody, is administered as a continuous i.v. infusion until the desired dose is achieved. In certain embodiments the MET agonist, for example anti-MET agonist antibody, is administered orally (per os).

EXAMPLES

The invention will be further understood with reference to the following non-limiting experimental examples.

Example 1: Generation of ant-MET agonist antibodies - Immunization of llamas

Immunizations of llamas and harvesting of peripheral blood lymphocytes (PBLs) as well as the subsequent extraction of RNA and amplification of antibody fragments were performed as described (De Haard et al., J. Bact. 187:4531-4541 , 2005). Two adult llamas *(Lama glama)* were immunized by intramuscular injection of a chimeric protein consisting of the extracellular domain (ECD) of human MET fused to the Fc portion of human IgG1 (MET-Fc; R&D Systems). Each llama received one injection per week for six weeks, for a total of

six injections. Each injection consisted in 0.2 mg protein in Freund's Incomplete Adjuvant in the neck divided over two spots.

Blood samples of 10 ml were collected pre- and post-immunization to investigate the immune response. Approximately one week after the last immunization, 400 ml of blood was collected and PBLs were obtained using the Ficoll-Paque method. Total RNA was extracted by the phenol-guanidine thiocyanate method (Chomczynski et al., Anal. Biochem. 162:1 56-159, 1987) and used as template for random cDNA synthesis using the SuperScriptTM III First-Strand Synthesis System kit (Life Technologies). Amplification of the cDNAs encoding the VH-CH1 regions of llama IgG1 and VL-CL domains (κ and λ) and subcloning into the phagemid vector pCB3 was performed as described (de Haard et al., J Biol Chem. 274:1821 8-18230, 1999). The E. coli strain TG1 (Netherland Culture Collection of Bacteria) was transformed using recombinant phagemids to generate 4 different Fab-expressing phage libraries (one λ and one κ library per immunized llama). Diversity was in the range of 10^8-10^9.

The immune response to the antigen was investigated by ELISA. To this end, we obtained the ECDs of human MET (UniProtKB # P08581 ; aa 1-932) and of mouse MET (UniProtKB # P16056.1 ; aa 1-931) by standard protein engineering techniques. Human or mouse MET ECD recombinant protein was immobilized in solid phase (100 ng/well in a 96-well plate) and exposed to serial dilutions of sera from llamas before (day 0) or after (day 45) immunization. Binding was revealed using a mouse anti-llama IgG1 (Daley et al., Clin. Vaccine Immunol. 12, 2005) and a HRP-conjugated donkey anti-mouse antibody (Jackson Laboratories). Both llamas displayed an immune response against human MET ECD. Consistent with the notion that the extracellular portion of human MET displays 87% homology with its mouse orthologue, a fairly good extent of cross-reactivity was also observed with mouse MET ECD.

Example 2: Selections and screenings of Fabs binding to both human and mouse MET

Fab-expressing phages from the libraries described above were produced according to standard phage display protocols. For selection, phages were first adsorbed to immobilized recombinant human MET ECD, washed, and then eluted using trypsin. After two cycles of selection with human MET ECD, two other cycles were performed in the same fashion using mouse MET ECD. In parallel, we also selected phages alternating a human MET ECD cycle with a mouse MET ECD cycle, for a total of four cycles. Phages selected by the two approaches were pooled together and then used to infect TG1 *E. coli.* Individual

colonies were isolated and secretion of Fabs was induced using IPTG (Fermentas). The Fab-containing periplasmic fraction of bacteria was collected and tested for its ability to bind human and mouse MET ECD by Surface Plasmon Resonance (SPR). Human or mouse MET ECD was immobilized on a CM-5 chip using amine coupling in sodium acetate buffer (GE Healthcare). The Fab-containing periplasmic extracts were loaded into a BIACORE 3000 apparatus (GE Healthcare) with a flow rate of 30 µl/min. The Fab off-rates (k_{off}) were measured over a two minute period. Binding of Fabs to human and mouse MET was further characterized by ELISA using MET ECD in solid phase and periplasmic crude extract in solution. Because Fabs are engineered with a MYC flag, binding was revealed using HRP-conjugated anti-MYC antibodies (ImTec Diagnostics).

Fabs that bound to both human and mouse MET in both SPR and ELISA were selected and their corresponding phages were sequenced (LGC Genomics). Cross-reactive Fab sequences were divided into families based on VH CDR3 sequence length and content. VH families were given an internal number not based on IMTG (International Immunogenetics Information System) nomenclature. Altogether, we could identify 11 different human/mouse cross-reactive Fabs belonging to 8 VH families. The CDR and FR sequences of heavy chain variable regions are shown in Table 3. The CDR and FR sequences of light chain variable regions are shown in Table 4. The full amino acid sequences of heavy chain and light chain variable regions are shown in Table 5. The full DNA sequences of heavy chain and light chain variable regions are shown in Table 6.

Table 3: Framework regions and CDR sequences for VH domains of Fabs binding to both human and mouse MET.

Clone	FR1	SEQ ID NO.	CDR1	SEQ ID NO.	FR2	SEQ ID NO.	CDR2	SEQ ID NO.	FR3	SEQ ID NO.	CDR3	SEQ ID NO.	FR4	SEQ ID NO.
76H10	QLQLVESG GGLVQPGG SLRVSCTA SGFTFN	1	TYYMT	2	WVRQAPG KGLEWVS	3	DINSGGG TYYADSV KG	4	RFTISRDNAKNT LYLQMNSLKPED TALYYCVR	5	VRIWPVG YDY	6	WGQGTQ VTVSS	7
71G3	QVQLVESG GGLVQPGG SLRVSCAA SGFTFS	8	TYYMS	9	WVRQAPG KGLEWVS	10	DIRTDGG TYYADSV KG	11	RFTMSRDNAKNT LYLQMNSLKPED TALYYCAR	12	TRIFPSG YDY	13	WGQGTQ VTVSS	14
71C3	QLQLVESG GGLVQPGG SLRLSCAA SGFTFS	15	SHAMS	16	WVRQAPG KGLEWVS	17	AINSGGG STSYADS VKG	18	RFTISRDNAKNT LYLQMNSLKPED TAVYYCAK	19	ELRFDLA RYTDYEA WDY	20	WGQGTQ VTVSS	21
71D4	ELQLVESG GGLVQPGG SLRLSCAA SGFTFS	22	GYGMS	23	WVRQAPG KGLEWVS	24	DINSGGG STSYADS VKG	25	RFTISRDNAKNT LYLQMNSLKPED TAVYYCAK	26	DMRLYLA RYNDYEA WDY	27	WGQGTQ VTVSS	28
71D6	ELQLVESG GGLVQPGG SLRLSCAA SGFTFS	29	SYGMS	30	WVRQAPG KGLEWVS	31	AINSYGG STSYADS VKG	32	RFTISRDNAKNT LYLQMNSLKPED TAVYYCAK	33	EVRADLS RYNDYES YDY	34	WGQGTQ VTVSS	35
71A3	EVQLVESG GGLVQPGG SLRLSCAA SGFSFK	36	DYDIT	37	WVRQAPG KGLEWVS	38	TITSRSG STSYVDS VKG	39	RFTISGDNAKNT LYLQMNSLKPED TAVYYCAK	40	VYATTWD VGPLGYG MDY	41	WGKGTL VTVSS	42

Clone	FR1	SEQ ID NO.	CDR1	SEQ ID NO.	FR2	SEQ ID NO.	CDR2	SEQ ID NO.	FR3	SEQ ID NO.	CDR3	SEQ ID NO.	FR4	SEQ ID NO.
71G2	EVQLQESG GGLVQPGG SLRLSCAA SGFTFS	43	IYDMS	44	WVRQAPG KGLEWVS	45	TINSDGS STSYVDS VKG	46	RFTISRDNAKNT LYLQMNSLKPED TAVYYCAK	47	VYGSTWD VGPMGYG MDY	48	WGKGTL VTVSS	49
76G7	QVQLVESG GNLVQPGG SLRLSCAA SGFTFS	50	NYYMS	51	WVRQAPG KGLEWVS	52	DIYSDGS TTWYSDS VKG	53	RFTISRDNAKNT LSLQMNSLKSED TAVYYCAR	54	VKIYPGG YDA	55	WGQGTQ VTVSS	56
71G12	QVQIQESG GDLVQPGG SLRVSCVV SGFTFS	57	RYYMS	58	WVRQAPG KGLEWVS	59	SIDSYGY STYYTDS VKG	60	RFTISRDNAKNT LYLQMNSLKPED TALYYCAR	61	AKTTWSY DY	62	WGQGTQ VTVSS	63
74C8	EVQLVESG GGLVQPGG SLRLSCAA SGFTFR	64	NYHMS	65	WVRQVPG KGFEWIS	66	DINSAGG STYYADS VKG	67	RFTISRDNAKNT LYLEMNSLKPED TALYYCAR	68	VNVWGVN Y	69	WGKGTL VSVSS	70
72F8	ELQLVESG GGLVQPGG SLRLSCAA SGFTFS	71	NYVMS	72	WVRQAPG KGLEWVS	73	DTNSGGS TSYADSV KG	74	RFTISRDNAKNT LYLQMNSLKPED TALYYCAR	75	SFFYGMN Y	76	WGKGTQ VTVSS	77

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Table 4 : Framework regions and CDR sequences for VL domains of Fabs binding to both human and mouse MET.

Clone	FR1	SEQ ID NO.	CDR1	SEQ ID NO.	FR2	SEQ ID NO.	CDR2	SEQ ID NO.	FR3	SEQ ID NO.	CDR3	SEQ ID NO.	FR4	SEQ ID NO.
76H10	QAVVTQEP SLSVSPGG TVTLTC	78	GLSSGSV TTSNYPG	79	WFQQTPGQ APRTLIY	80	NTNNRHS	81	GVPSRFSGSISG NKAALTITGAQP EDEADYYC	82	SLYTGS YTTV	83	FGGGTH LTVL	84
71G3	QAVVTQEP SLSVSPGG TVTLTC	85	GLSSGSV TTSNYPG	86	WFQQTPGQ APRTLIY	87	NTNSRHS	88	GVPSRFSGSISG NKAALTIMGAQP EDEADYYC	89	SLYPGS TTV	90	FGGGTH LTVL	91
71C3	SYELTQPS ALSVTLGQ TAKITC	92	QGGSLGS SYAH	93	WYQQKPGQ APVLVIY	94	DDDSRPS	95	GIPERFSGSSSG GTATLTISGAQA EDEGDYYC	96	QSADSS GNAAV	97	FGGGTH LTVL	98
71D4	SSALTQPS ALSVTLGQ TAKITC	99	QGGSLGS SYAH	100	WYQQKPGQ APVLVIY	101	DDDSRPS	102	GIPERFSGSSSG GTATLTISGAQA EDEGDYYC	103	QSADSS GNAAV	104	FGGGTH LTVL	105
71D6	QPVLNQPS ALSVTLGQ TAKITC	106	QGGSLGA RYAH	107	WYQQKPGQ APVLVIY	108	DDDSRPS	109	GIPERFSGSSSG GTATLTISGAQA EDEGDYYC	110	QSADSS GSV	111	FGGGTH LTVL	112
71A3	SYELTQPS ALSVTLGQ TAKITC	113	QGGSLGS SYAH	114	WYQQKPGQ APVLVIY	115	DDDSRPS	116	GIPERFSGSSSG GTATLTISGAQA EDEGDYYC	117	QSADSS GNAAV	118	FGGGTH LTVL	119
71G2	SSALTQPS ALSVSLGQ TARITC	120	QGGSLGS SYAH	121	WYQQKPGQ APVLVIY	122	GDDSRPS	123	GIPERFSGSSSG GTATLTISGAQA EDEDDYYC	124	QSTDSS GNTV	125	FGGGTR LTVL	126
76G7	QAGLTQPP SVSGSPGK TVTISC	127	AGNSSDV GYGNYVS	128	WYQQFPGM APKLLIY	129	LVNKRAS	130	GITDRFSGSKSG NTASLTISGLQS EDEADYYC	131	ASYTGS NNIV	132	FGGGTH LTVL	133

Clone	FR1	SEQ ID NO.	CDR1	SEQ ID NO.	FR2	SEQ ID NO.	CDR2	SEQ ID NO.	FR3	SEQ ID NO.	CDR3	SEQ ID NO.	FR4	SEQ ID NO.
71G12	EIVLTQSP SSVTASVG GKVTINC	134	KSSQSVF IASNQKT YLN	135	WYQQRPGQ SPRLVIS	136	YASTRES	137	GIPDRFSGSGSGST TDFTLTISSVQP EDAAVYYC	138	QQAYSH PT	139	FGQGTK VELK	140
74C8	QTVVTQEP SLSVSPGG TVTLTC	141	GLSSGSV TTSNYPG	142	WFQQTPGQ APRTLIY	143	NTNSRHS	144	GVPSRFSGSISG NKAALTITGAQP EDEADYYC	145	SLYPGS YTNV	146	FGGGTH LTVL	147
72F8	QSALTQPP SLSASPGS SVRLTC	148	TLSSGNN IGSYDIS	149	WYQQKAGS PPRYLLN	150	YYTDSRK HQDS	151	GVPSRFSGSKDA SANAGLLLISGL QPEDEADYYC	152	SAYKSG SYRWV	153	FGGGTH VTVL	154

Table 5: Variable domain amino acid sequences of Fabs binding to both human and mouse MET.

CLONE	VH	SEQ ID NO.	VL	SEQ ID NO.
76H10	QLQLVESGGGLVQPGGSLRVSCTASGFTFNTYYMTWVR QAPGKGLEWVSDINSGGGTYYADSVKGRFTISRDNAKN TLYLQMNSLKPEDTALYYCVRVRIWPVGYDYWGQGTQV TVSS	155	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTTSNYPGWF QQTPGQAPRTLIYNTNNRHSGVPSRFSGSISGNKAALT ITGAQPEDEADYCSLYTGSYTTVFGGGTHLTVL	156
71G3	QVQLVESGGGLVQPGGSLRVSCAASGFTFSTYYMSWVR QAPGKGLEWVSDIRTDGGTYYADSVKGRFTMSRDNAKN TLYLQMNSLKPEDTALYYCARTRIFPSGYDYWGQGTQV TVSS	157	QAVVTQEPSLSVSPGGTVTLTCGLSSGSVTTSNYPGWF QQTPGQAPRTLIYNTNSRHSGVPSRFSGSISGNKAALT IMGAQPEDEADYCSLYPGSTTVFGGGTHLTVL	158
71C3	QLQLVESGGGLVQPGGSLRLSCAASGFTFSSHAMSWVR QAPGKGLEWVSAINSGGGTSYADSVKGRFTISRDNAK NTLYLQMNSLKPEDTAVYYCAKELRFDLARYTDYEAWD YWGQGTQVTVSS	159	SYELTQPSALSVTLGQTAKITCQGGSLGSSYAHWYQQK PGQAPVLVIYDDDSRPSGIPERFSGSSSGTATLTISG AQAEDEGDYYCQSADSSGNAAVFGGGTHLTVL	160
71D4	ELQLVESGGGLVQPGGSLRLSCAASGFTFSGYGMSWVR QAPGKGLEWVSDINSGGGTSYADSVKGRFTISRDNAK NTLYLQMNSLKPEDTAVYYCAKDMRLYLARYNDYEAWD YWGQGTQVTVSS	161	SSALTQPSALSVTLGQTAKITCQGGSLGSSYAHWYQQK PGQAPVLVIYDDDSRPSGIPERFSGSSSGTATLTISG AQAEDEGDYYCQSADSSGNAAVFGGGTHLTVL	162
71D6	ELQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVR QAPGKGLEWVSAINSYGGSTSYADSVKGRFTISRDNAK NTLYLQMNSLKPEDTAVYYCAKEVRADLSRYNDYESYD YWGQGTQVTVSS	163	QPVLNQPSALSVTLGQTAKITCQGGSLGARYAHWYQQK PGQAPVLVIYDDDSRPSGIPERFSGSSSGTATLTISG AQAEDEGDYYCQSADSSGSVFGGGTHLTVL	164
71A3	EVQLVESGGGLVQPGGSLRLSCAASGFSFKDYDITWVR QAPGKGLEWVSTITSRSGSTSYVDSVKGRFTISGDNAK NTLYLQMNSLKPEDTAVYYCAKVYATTWDVGPLGYGMD YWGKGTLVTVSS	165	SYELTQPSALSVTLGQTAKITCQGGSLGSSYAHWYQQK PGQAPVLVIYDDDSRPSGIPERFSGSSSGTATLTISG AQAEDEGDYYCQSADSSGNAAVFGGGTHLTVL	166

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CLONE	VH	SEQ ID NO.	VL	SEQ ID NO.
71G2	EVQLQESGGGLVQPGGSLRLSCAASGFTFSIYDMSWVRQAPGKGLEWVSTINSDGSTSYVDSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAKVYGSTWDVGPMGYGMDYWGKGTLVTVSS	167	SSALTQPSALSVSLGQTARITCQGGSLGSSYAHWYQQKPGQAPVLVIYGDDSRPSGIPERFSGSSSGGTATLTISGAQAEDEDDYYCQSTDSSGNTVFGGGTRLTVL	168
76G7	QVQLVESGGNLVQPGGSLRLSCAASGFTFSNYYMSWVRQAPGKGLEWVSDIYSDGSTTWYSDSVKGRFTISRDNAKNTLSLQMNSLKSEDTAVYYCARVKIYPGGYDAWGQGTQVVTVSS	169	QAGLTQPPSVSGSPGKTVTISCAGNSSDVGYGNYVSWYQQFPGMAPKLLIYLVNKRASGITDRFSGSKSGNTASLTISGLQSEDEADYYCASYTGSNNIVFGGGTHLTVL	170
71G12	QVQLQESGGDLVQPGGSLRVSCVVSGFTFSRYYMSWVRQAPGKGLEWVSSIDSYGYSTYYTDSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARAKTTWSYDYWGQGTQVTVSS	171	EIVLTQSPSSVTASVGGKVTINCKSSQSVFIASNQKTYLNWYQQRPGQSPRLVISYASTRESGIPDRFSGSGSTTDFTLTISSVQPEDAAVYYCQQAYSHPTFGQGTKVELK	172
74C8	EVQLVESGGGLVQPGGSLRLSCAASGFTFRNYHMSWVRQVPGKGFEWISDINSAGGSTYYADSVKGRFTISRDNAKNTLYLEMNSLKPEDTALYYCARVNVWGVNYWGKGTLVSVSS	173	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTTSNYPGWFQQTPGQAPRTLIYNTNSRHSGVPSRFSGSISGNKAALTITGAQPEDEADYYCSLYPGSYTNVFGGGTHLTVL	174
72F8	ELQLVESGGGLVQPGGSLRLSCAASGFTFSNYVMSWVRQAPGKGLEWVSDTNSGGSTSYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARSFFYGMNYWGKGTQVTVSS	175	QSALTQPPSLSASPGSSVRLTCTLSSGNNIGSYDISWYQQKAGSPPRYLLNYYTDSRKHQDSGVPSRFSGSKDASANAGLLLISGLQPEDEADYCSAYKSGSYRWVFGGGTHVTVL	176

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Table 6: Variable domain nucleotide sequences of Fabs binding to both human and mouse MET.

Clone	VH	SEQ ID NO.	VL	SEQ ID NO.
76H10	CAGTTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGAGTTTCCTGTACAGCCTCTGCGATTCACCTTCAATACCTACTACATGACCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTCGAGTGGGTCTCAGATATTAATAGTGGTGGTACATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTATATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCCCTGTATTACTGTGTAAGAGTTCGTATTTGGCCAGTGGGGATATGACTACTGGGGCCAGGGGACCCAGGTCACCGTTTCCTCA	177	CAGGCTGTGGTGACCCAGGAGCCGTCCCTGTCAGTGTCTCCAGGAGGACGGGTCACACTCACCTGCGCGCCTCAGCTCTGGGTCTGTCACTACCAGTAACTACCCTGGTTGGTTCCAGCAGACACCGGGCCAGGCTCCACTCTTATCTACAACACAAACAACCGCCACTCTGGGGTCCCCAGTCGCTTCTCCGGATCCATCTCTGGGAACAAAGCCGGCCCCTCACCATCACGGGGCCCAGCCCGAGGACGAGGCCGACTATTACTGTTCTCTATATACTGGCAGTTACACTACTGTGTTCGGCGGAGGGACCCATCTGACCGTCCTG	178
71G3	CAGGTGCAGCTCGTGCAGTCTGGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGAGTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGTTACTACATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTCGAGTGGGTCTCAGATATTCGTACTGATGGTGGCACATGTCCAGAGACTATTGCAGACTCCGTGAAGGGCCGATTCACCATGTCCAGAGACAACGCCAAGAACACGCTGTATCTACAAATGAACAGCCTGAGAACTCGAATTTTCCCACGGCGCCCTGTATTACTGTGTGCAAGAACTCGGGTATGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	179	CAGGCTGTGGTGACCCAGGAGCCGTCCCTGTCAGTGTCTCCAGGAGGACGGGTCACACTCACCTGCGCGCCTCAGCTCTGGGTCTGTCACTACCAGTAACTACCCTGGTTGGTTCCAGCAGACACCGGGCCAGGCTCCACTCTTATCTACAACACAAACAACCGCCACTCTGGGGTCCCCAGTCGCTTCTCCGGATCCATCTCTGGGAACAAAGCCGGCCCCTCACCATCATGGGGGCCCAGCCCGAGGACGAGGCCGACTATTACTGTTCTCTGTACCCTGGTAGTAGTACCACTGTGTTCGGCGGAGGGACCCATCTGACCGTCCTG	180

Clone	VH	SEQ ID NO.	VL	SEQ ID NO.
71C3	CAGTTGCAGCTGGTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCCATGCCATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTCGAGTGGGTCTCAGCTATTAATAGTGGTGGTGGTAGCACAAGCTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTACCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCGTGTATTACTGTGCAAAAGAGCTGAGATTCGACCTAGCAAGGTATACCGACTATGAGGCCTGGGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	181	TCCTATGAGCTGACTCAGCCCCTCCGCGCTGTCCGTAACCTTGGGACAGACGGCCAAGATCACCTGCCAAGGTGGCAGCTTAGGTAGCAGTTATGCTCACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCATCTATGATGATGACAGCAGGCCCTCAGGGATCCCTGAGCGGTTCTCTGGCTCCAGCTCTGGGGGCCACAGCAGCCTGACCATCAGCGGGGCCCAGGCCGAGGACGAGGGTGACTATTACTGTCAGTCAGCAGACAGCAGTGGTAATGCTGCTGTGTTCGGCGGAGGGACCCATCTGACCGTCCTG	182
71D4	GAGTTGCAGCTGGTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTGGCTATGGCATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTCGAGTGGGTCTCAGATATTAATAGTGGTGGTGGTAGCACAAGCTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCGTGTATTACTGTGCAAAAGATATGAGATTATACCTAGCAAGGTATAAACGACTATGAGGCCTGGGACTACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	183	TCCTCTGCACTGACTCAGCCCCTCCGCGCTGTCCGTAACCTTGGGACAGACGGCCAAGATCACCTGCCAAGGTGGCAGCTTAGGTAGCAGTTATGCTCACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCATCTATGATGATGACAGCAGGCCCTCAGGGATCCCTGAGCGGTTCTCTGGCTCCAGCTCTGGGGGCCACAGCAGCCTGACCATCAGCGGGGCCCAGGCCGAGGACGAGGGTGACTATTACTGTCAGTCAGCAGACAGCAGTGGTAATGCTGCTGTGTTCGGCGGAGGGACCCATCTGACCGTCCTG	184

Clone	VH	SEQ ID NO.	VL	SEQ ID NO.
71D6	GAGGTTGCAGCTGGTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGAGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTCTCAGCTATTAATAGTTATGGTGGTAGCACAAGCTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAAAACCTGAGGACACGGCCGTGTATTACTGTGCAAAAGAAGTGCGGGCCGACCTAAGCCGCTATAAACGACTATGAGTCGTATGACTACTGGGGCCAGGGGACCCCAGGTCACCGTCTCCTCA	185	CAGCCGGTGCTGCTGAATCAGCCCTCCGCGCTGTCCGTAACCTTGGGACAGACGGCCAAGATCACCTGCCAAGGTGGCAGCTTAGGTGCGCGTTATGCTCACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCATCTATGATGATGACAGCAGGCCCTCAGGGATCCCTGAGCGGGTTCTCTGGCTCCAGCTCTGGGGGCCACAGCAGCCCTGACCACCTCAGCGGGGCCCAGGCCGAGGACGAGGGTGACTATTACTGTCAGTCAGCAGACAGCAGTGGTTCTGTGTTCGGCGGAGGGACCCATCTGACCGTCCTG	186
71A3	GAGGTTGCAGCTCGTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCAGCTTCAAGGACTATGACATAACCTGGGTCCGCCAGGCTCCGGGAAAGGGGCTCGAGTGGGTCTCAACTATTACTAGTCGTAGTGGTAGCACAAGCTATGTAGACTCCGTAAAGGGCCGATTCACCATCTCCGGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAAAAGTTTACGCGAGGACACGGCCGTGTATTACTGTGCAAAAGTTTACGGACGACTACCTGGGACGTCGGCCCCTCTGGGCTGGGTCTCAACTATTACTGGGGCCAAGGGGACACCCTGGTCACCGTCTCCTCA	187	TCCTATGAGCTGACTCAGCCCTCCGCGCTGTCCGTAACCTTGGGACAGACGGCCAAGATCACCTGCCAAGGTGGCAGCTTAGGTAGCAGTTATGCTCACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCATCTATGATGATGACAGCAGGCCCTCAGGGATCCCTGAGCGGGTTCTCTGGCTCCAGCTCTGGGGGCCACAGCAGCCCTGACCATCAGCGGGGCCCAGGCCGAGGACGAGGGTGACTATTACTGTCAGTCAGCAGACAGCAGTGGTAATGCTGTGTTCGGCGGGAGGGACCCCATCTGACCGTCCTG	188

Clone	VH	SEQ ID NO.	VL	SEQ ID NO.
71G2	GAGGTGCAGCTGCAGGAGTCGGGGGGAGGCTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTATATATGACATGAGCTGGTCCGCCAGGCTCCAGGGAAAGGGGCTCGAGTGGGTCTCAACTATTAATAGTGATGGTAGTAGCACAAGCTATGTAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCCGTGTATTACTGTGCGAAAGTTTACGGTAGTACCTGGGACGTCGGCCCTATGGGCTACGGCATGGACTACTGGGGCCAAAGGGACACCCTGGTCACTGTCTCCTCA	189	TCCTCTGCACTGACTCAGCCCTCCGCGCTGTCCGTGTCCTTGGGACAGACGGCCAGGATCACCTGCCAAGGTGGCAGCTTAGGTAGCAGTTATGCTCACTGGTACCAGCAGAAGCCAGGCCAGGCCCCTGTGCTGGTCATCTATGGTGATGACAGCAGGCCCTCAGGGATCCCTGAGCGGGTTCTCTGGCTCCAGCTCTGGGGGCACAGCCACCCTGACCATCAGCGGGGCCCAGGCCGAGGACGAGGATGACTATTACTGTCAGTCAACAGACAGCAGTGGTAATACTGTGTTCGGCGGAGGGACCCGACTGACCGTCCTG	190
76G7	CAGGTGCAGCTGGTGGAGTCTGGGGGAAACTTGGTGCAGCCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAACTACTACATGAGCTGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGGTGTCCGATATTTATAGTGACGGTAGTACCACCATCTCCAGAGACAACGCCAAGAACACGCTGTCTCTGCAAATGAACAGTCTGAAATCTGAGGACACGGCCGTGTCTGCGCGCGCCTGGGGCCAGGGGACCCCAGATCCGGGGGGTATGACGCCCTGGGGCCTGGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA	191	CAGGCAGGGCTGACTCAGCCTCCCTCCGTGTCTGGGTCTCCAGGAAAGACGGTCACCATCTCCTGTGCAGGAAACAGCAGTGATGTTGGGTATGGAAACTATGTCTCCTGTACCAGCAGTTCCCAGGAATGGCCCCCAAACTCCTGATATATCTCGTCAATAAACGGGCCCTCAGGGATCACTGATCGCTTCTCTGGCTCCAAGTCAGGCAACACGGCCTCCCTGACCATCTCTGGGCTCCAGTCTGAGGACGAGGCTGATTATTACTGTGCCTCATATACACAGGTAGCAACAATATCGTGTTCGGCGGAGGGACCCCATCTAACCGTCCTC	192

Clone	VH	SEQ ID NO.	VL	SEQ ID NO.
71G12	CAGGTGCAGCTGCAGGAGTCGGGGGGAGACTTGGTGCAGCCTGGGGGGTCTCTGAGAGTCTCCTGTGTAGTCTCGCGATTCACCTTCAGTGCTACTACATGAGCTGGTCCGCCAGGCTCCAGGGAAGGGGCTCGAGTGGGTCTCATCTATTGATAGTTATGGTTACAGCACATCTCCAGAGACACAGCCAAGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAAACCGAGGACACGGCCCTGTATTACTGTGGGGGCCAGGGGACCGACTTGGGAGTTATGACTACTGGGGCCAAGGGGACCCCAGGTCACCGTCTCCTCA	193	GAAATTGTGTTGACGCAGTCTCCCAGCTCCGTGACTGCATCTGTAGGAGGGAAGGTCACTATCAACTGTAAGTCCAGCCAGAGCGTCTTCATAGCTTCTAATCAGAAAAACCTACTTAAACTGGTACCAGCAGAGACCTGGACAGTCTCCGAGGTTGGTCATCAGCTATGCGTCCACCCGTGAATCGGGGATCCCTGATCGATTCAGCGGCAGTGGGTCCACAACAGATTTCACTCTCACGATCAGCAGTGTCCAGCCTGAAGATGCGGCCGTGTATTACTGTCAGCAGCTTATAGCCATCCAACGTTCGGCCCAGGGGACCAAGGTGGAACTCAAA	194
74C8	GAGGTGCAGCTCGTGGAGTCTGGGGGAGGCTTGGTGCAACCTGGGGGGTCTCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGGAATTACCACATGAGTTGGGTCCGCCAGGTTCCAGGGAAGGGGTTCGAGTGGGATCTCAGATATTAATAGTGCAGGTGGTAGCACATCTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAAAGTCTCGGACACGGCCCTGTATTACTGTGGGGGCAAAGGGGACCCTGGTCAGCGTCTCCTCA	195	CAGACTGTGGTGACTCAGGAGCCGTCCCTGTCAGTGTCTCCAGGAGGGACGGTCACACTCACCTGCGCCTCAGCTCTGGGTCTGTCACTACCAGTAACTACCCTGGTTGGTTCCAGCAGACACCAGGCCAGGCTCCACTCGGGTCCCCAGTCGCTCAACACAAACAGCGCCGCCACTCTGGGGGTCCCCTCACCTCTCCGGATCCATCTCTGGGAACAAAGCCGCCCTCACCATCACGGGGCCCAGCCCGAGGACGAGGCCGACTATTACTGTTCTCTGTACCCTGGTAGTTACACTAATGTGTTCGGCGGAGGGACCCCATCTGACCGGTCCTG	196

Clone	VH	SEQ ID NO.	VL	SEQ ID NO.
72F8	GAGTTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTGCAGCCTGgGGGGTCTCTGAGACTCTcCTGTGCAGCCTCTGGATTCACCTTCAGCAACTATGTCATGAGCTGGGTCCGCCAGGCTCCAGGGAAAGGGCTCGAGTGGGTCTCAGATACTAATAGTGGTGGTAGCACAAGCTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCTAGAGACAACGCCAAGAACACGCTGTATTTGCAAATGAACAGCCTGAGAACCTGAGGACACGGCCATTGTATTACTGTGCGAGATCATTTTTCTACGGCATGAACTACTGGGGCCAAAGGGACCCAGGTCACCGTGTCCTCA	197	CAGTCTGCCCTGACTCAGCCGCGCCCTCCCTCTCTGCATCTCCGGGATCATCTGTCAGACTCACCCTGCACCCTGAGCAGTGGAAACAATATTGGCAGCTATGACATAAGTTGGTACCAGCAGAAGGCAGGGAGCCCTCCCCGTACCTCCTGAACTACTACACCGACTCACGCAAGCACCAGGACTCCGGGGTCCCGAGCCGCCTTCTCTGCTCAAAGATGCCTCGGCCAACGCAGGGCTTCTGCCTTCATCTCTGGGCTTCAGCCCCGAGGACGAGGCTGACTATTACTGTTCTGCATACAAGAGTGGTTCTTACCGTTGGGGTGTTCGGCGGAGGGACCGCACGTGACCGTCCTG	198

The various Fab families and their ability to bind human and mouse MET are shown in **Table 7.**

Table 7: Fabs binding to both human MET (hMET) and mouse MET (mMET). Fabs are grouped in families based on their VH CDR3 sequence. Binding of Fabs to human and mouse MET ECD was determined by Surface Plasmon Resonance (SPR) and by ELISA. SPR values represent the koff (s^{-1}). ELISA values represent the Optical Density (OD) at 450 nm (AU, arbitrary units). Both SPR and ELISA were performed using crude periplasmic extracts. Fab concentration in the extract was not determined. Values are the mean of three independent measurements.

Fab	VH	VL	SPR (K_{off}; s^{-1})		ELISA (OD$_{450}$; AU)	
			hMET	mMET	hMET	mMET
76H10	VH 1	Lambda	5.68E-03	5.44E-03	3.704	3.697
71G3	VH 2	Lambda	1.42E-03	1.41E-03	3.462	3.443
71D6	VH 3a	Lambda	2.94E-03	2.67E-03	3.261	3.072
71C3	VH 3b	Lambda	2.25E-03	2.58E-03	1.650	1.643
71D4	VH 3c	Lambda	2.17E-03	2.38E-03	0.311	0.307
71A3	VH 4	Lambda	4.92E-03	4.74E-03	0.581	0.524
71G2	VH 4	Lambda	1.21E-03	1.48E-03	0.561	0.543
76G7	VH 5	Lambda	4.32E-03	4.07E-03	3.199	3.075
71G12	VH 6	Kappa	2.28E-03	2.55E-03	0.450	0.420
74C8	VH 9	Lambda	3.48E-03	3.70E-03	2.976	2.924
72F8	VH 10	Lambda	4.96E-03	4.58E-03	3.379	3.085

Example 3: Chimerization of Fabs into mAbs

The cDNAs encoding the VH and VL (κ or λ) domains of selected Fab fragments were engineered into two separate pUPE mammalian expression vectors (U-protein Express) containing the cDNAs encoding CH1, CH2 and CH3 of human IgG1 or the human CL (κ or λ), respectively.

Production (by transient transfection of mammalian cells) and purification (by protein A affinity chromatography) of the resulting chimeric llama-human IgG1 molecules was outsourced to U-protein Express. Binding of chimeric mAbs to MET was determined by ELISA using hMET or mMET ECD in solid phase and increasing concentrations of antibodies (0-20 nM) in solution. Binding was revealed using HRP-conjugated anti-human Fc antibodies (Jackson Immuno Research Laboratories). This analysis revealed that all chimeric llama-human antibodies bound to human and mouse MET with picomolar affinity,

displaying an EC_{50} comprised between 0.06 nM and 0.3 nM. Binding capacity (E_{MAX}) varied from antibody to antibody, possibly due to partial epitope exposure in the immobilized antigen, but was similar in the human and mouse setting. EC_{50} and E_{MAX} values are shown in **Table 9.**

5

Table 9: Binding of chimeric mAbs to human and mouse MET as determined by ELISA using immobilized MET ECD in solid phase and increasing concentrations (0-20 nM) of antibodies in solution. $EC_{5}0$ values are expressed as nMol/L. **E_{MAX}** values are expressed as Optical Density (OD) at 450 nm (AU, arbitrary units).

mAb	hMET		mMET	
	EC_{50}	E_{MAX}	EC_{50}	E_{MAX}
76H10	0.090	2.669	0.062	2.662
71G3	0.067	2.835	0.057	2.977
71D6	0.026	2.079	0.049	2.009
71C3	0.203	2.460	0.293	2.238
71D4	0.207	1.428	0.274	1.170
71A3	0.229	2.401	0.176	2.730
71G2	0.112	3.094	0.101	3.168
76G7	0.128	2.622	0.103	2.776
71G12	0.106	3.076	0.127	2.973
74C8	0.090	0.994	0.116	0.896
72F8	0.064	2.779	0.048	2.903

10

We also analysed whether chimeric anti-MET antibodies bound to native human and mouse MET in living cells. To this end, increasing concentrations of antibodies (0-100 nM) were incubated with A549 human lung carcinoma cells (American Type Culture Collection) or MLP29 mouse liver precursor cells (a gift of Prof. Enzo Medico, University of Torino, Strada Provinciale 142 km 3.95, Candiolo, Torino, Italy; Medico et al., Mol Biol Cell 7, 495-504, 1996), which both express physiological levels of MET. Antibody binding to cells was analysed by flow cytometry using phycoerythrin-conjugated anti-human IgG1 antibodies (eBioscience) and a CyAn ADP analyser (Beckman Coulter). As a positive control for human MET binding, we used a commercial mouse anti-human MET antibody (R&D Systems) and phycoerythrin-conjugated anti-mouse IgG1 antibodies (eBioscience). As a positive control for mouse MET binding we used a commercial goat anti-mouse MET antibody (R&D Systems) and phycoerythrin-conjugated anti-goat IgG1 antibodies (eBioscience). All antibodies displayed dose-dependent binding to both human and mouse cells with an EC_{50} varying between 0.2 nM and 2.5 nM. Consistent with the data

obtained in ELISA, maximal binding (E_{MAX}) varied depending on antibody, but was similar in human and mouse cells. These results indicate that the chimeric llama-human antibodies recognize membrane-bound MET in its native conformation in both human and mouse cellular systems. EC_{50} and E_{MAX} values are shown in **Table 10.**

5

Table 10: Binding of chimeric mAbs to human and mouse cells as determined by flow cytometry using increasing concentrations (0-50 nM) of antibodies. EC_5o values are expressed as nMol/L. E_{MAX} values are expressed as % relative to control.

mAb	Human cells (A549)		Mouse cells (MLP29)	
	EC_{50}	E_{MAX}	EC_{50}	E_{MAX}
76H10	2.345	130.2	1.603	124.3
71G3	0.296	116.9	0.214	116.2
71D6	0.259	112.7	0.383	121.2
71C3	0.572	106.5	0.585	115.1
71D4	0.371	107.2	0.498	94.8
71A3	0.514	160.8	0.811	144.2
71G2	0.604	144.4	0.688	129.9
76G7	2.298	121.2	2.371	114.8
71G12	2.291	109.9	2.539	121.2
74C8	0.235	85.7	0.208	73.8
72F8	0.371	156.3	0.359	171.6

10

Example 4: Receptor regions responsible for antibody binding

In order to map the receptor regions recognized by antibodies binding to both human and mouse MET (herein after referred to as human/mouse equivalent anti-MET antibodies), we measured their ability to bind to a panel of engineered proteins derived from human MET generated as described (Basilico et al, J Biol. Chem. 283, 21267-21227, 2008). This panel included: the entire MET ECD (Decoy MET); a MET ECD lacking IPT domains 3 and 4 (SEMA-PSI-IPT 1-2); a MET ECD lacking IPT domains 1-4 (SEMA-PSI); the isolated SEMA domain (SEMA); a fragment containing IPT domains 3 and 4 (IPT 3-4). Engineered MET proteins were immobilized in solid phase and exposed to increasing concentrations of chimeric antibodies (0-50 nM) in solution. Binding was revealed using HRP-conjugated anti-human Fc antibodies (Jackson Immuno Research Laboratories). As shown in **Table 11,** this analysis revealed that 7 mAbs recognize an epitope within the SEMA domain, while the other 4 recognize an epitope within the PSI domain.

Table 11: Binding of human/mouse equivalent anti-MET antibodies to the panel of MET deletion mutants. The MET domain responsible for antibody binding is indicated in the last column to the right.

mAb	Decoy MET	SEMA-PSI-IPT 1-2	SEMA-PSI	SEMA	IPT 3-4	Binding domain
76H10	+	+	+	−	−	PSI
71G3	+	+	+	−	−	PSI
71D6	+	+	+	+	−	SEMA
71C3	+	+	+	+	−	SEMA
71D4	+	+	+	+	−	SEMA
71A3	+	+	+	+	−	SEMA
71G2	+	+	+	+	−	SEMA
76G7	+	+	+	−	−	PSI
71G12	+	+	+	−	−	PSI
74C8	+	+	+	+	−	SEMA
72F8	+	+	+	+	−	SEMA

5   To more finely map the regions of MET responsible for antibody binding, we exploited the absence of cross-reactivity between our antibodies and llama MET (the organism used for generating these immunoglobulins). To this end, we generated a series of llama-human and human-llama chimeric MET proteins spanning the entire MET ECD as described (Basilico et al., J Clin Invest. 124, 3172-31 86, 2014). Chimeras were

10   immobilized in solid phase and then exposed to increasing concentrations of mAbs (0-20 nM) in solution. Binding was revealed using HRP-conjugated anti-human Fc antibodies (Jackson Immuno Research Laboratories). This analysis unveiled that 5 SEMA-binding mAbs (71 D6, 71C3, 71D4, 71A3, 71G2) recognize an epitope localized between aa 314-372 of human MET, a region that corresponds to blades 4-5 of the 7-bladed SEMA β-

15   propeller (Stamos et al., EMBO J. 23, 2325-2335, 2004). The other 2 SEMA-binding mAbs (74C8, 72F8) recognize an epitope localized between aa 123-223 and 224-31 1, respectively, corresponding to blades 1-3 and 1-4 of the SEMA β-propeller. The PSI-binding mAbs (76H10, 71G3, 76G7, 71G1 2) did not appear to display any significant binding to any of the two PSI chimeras. Considering the results presented in **Table 11,**

20   these antibodies probably recognize an epitope localized between aa 546 and 562 of human MET. These results are summarized in **Table 12.**

Table 12: Mapping of the epitopes recognized by human/mouse equivalent anti-MET antibodies as determined by ELISA. Human MET ECD (hMET) or llama MET ECD (lMET) as well as the llama-human MET chimeric proteins (CH1-7) were immobilized in solid phase and then exposed to increasing concentrations of mAbs.

mAb	hMET	lMET	CH1	CH2	CH3	CH4	CH5	CH6	CH7	Epitope (aa)
76H10	+	–	+	+	+	+	+	–	–	546-562
71G3	+	–	+	+	+	+	+	–	–	546-562
71D6	+	–	+	+	+	–	–	+	+	314-372
71C3	+	–	+	+	+	–	–	+	+	314-372
71D4	+	–	+	+	+	–	–	+	+	314-372
71A3	+	–	+	+	+	–	–	+	+	314-372
71G2	+	–	+	+	+	–	–	+	+	314-372
76G7	+	–	+	+	+	+	+	–	–	546-562
71G12	+	–	+	+	+	+	+	–	–	546-562
74C8	+	–	+	–	–	–	–	+	+	123-223
72F8	+	–	+	+	–	–	–	+	+	224-311

Example 5: HGF competition assays

The above analysis suggests that the epitopes recognized by some of the human/mouse equivalent anti-MET antibodies may overlap with those engaged by HGF when binding to MET (Stamos et al., EMBO J. 23, 2325-2335, 2004; Merchant et al., Proc Natl Acad Sci USA 110, E2987-2996, 2013; Basilico et al., J Clin Invest. 124, 3172-3186, 2014). To investigate along this line, we tested the competition between mAbs and HGF by ELISA. Recombinant human and mouse HGF (R&D Systems) were biotinylated at the N-terminus using NHS-LC-biotin (Thermo Scientific). MET-Fc protein, either human or mouse (R&D Systems), was immobilized in solid phase and then exposed to 0.3 nM biotinylated HGF, either human or mouse, in the presence of increasing concentrations of antibodies (0-120 nM). HGF binding to MET was revealed using HRP-conjugated streptavidin (Sigma-Aldrich). As shown in **Table 13,** this analysis allowed to divide human/mouse equivalent anti-MET mAbs into two groups: full HGF competitors (71D6, 71C3, 71D4, 71A3, 71G2), and partial HGF competitors (76H10, 71G3, 76G7, 71G12, 74C8, 72F8).

Table 13: Ability of human/mouse equivalent anti-MET antibodies to compete with HGF for binding to MET as determined by ELISA. A MET-Fc chimeric protein (either human or mouse) was immobilized in solid phase and exposed to a fixed concentration of biotinylated HGF (either human or mouse), in the presence of increasing concentrations of antibodies. HGF binding to MET was revealed using HRP-conjugated streptavidin. Antibody-HGF competition is expressed as IC_{50} (the concentration that achieves 50% competition) and I_{MAX} (the maximum % competition reached at saturation).

mAb	hHGF on hMET		mHGF on mMET	
	IC_{50} (nM)	I_{MAX} (%)	IC_{50} (nM)	I_{MAX} (%)
76H10	1.86	64.22	2.01	62.71
71G3	0.49	63.16	0.53	62.87
71D6	0.29	98.34	0.34	90.54
71C3	1.42	93.64	1.56	89.23
71D4	0.34	95.62	0.40	91.34
71A3	0.51	93.37	0.54	87.74
71G2	0.23	97.84	0.26	91.86
76G7	1.47	69.42	1.56	62.52
71G12	3.87	51.39	4.05	50.67
74C8	0.43	76.89	0.49	71.55
72F8	0.45	77.34	0.52	72.79

As a general rule, SEMA binders displaced HGF more effectively than PSI binders. In particular, those antibodies that recognize an epitope within blades 4 and 5 of the SEMA β-propeller were the most potent HGF competitors (71D6, 71C3, 71D4, 71A3, 71G2). This observation is consistent with the notion that SEMA blade 5 contains the high affinity binding site for the α-chain of HGF (Merchant et al., Proc Natl Acad Sci USA 110, E2987-2996, 2013). The PSI domain has not been shown to participate directly with HGF, but it has been suggested to function as a 'hinge' regulating the accommodation of HGF between the SEMA domain and the IPT region (Basilico et al., J Clin Invest. 124, 3172-3186, 2014). It is therefore likely that mAbs binding to PSI (76H10, 71G3, 76G7, 71G12) hamper HGF binding to MET by interfering with this process or by steric hindrance, and not by direct competition with the ligand. Finally, blades 1-3 of the SEMA β-propeller have been shown to be responsible for low-affinity binding of the β-chain of HGF, which plays a central role in MET activation but only partially contributes to the HGF-MET binding strength (Stamos et al., EMBO J. 23, 2325-2335, 2004). This could explain why mAbs binding to that region of MET (74C8, 72F8) are partial competitors of HGF.

Example 6: MET activation assays

Due to their bivalent nature, immunoglobulins directed against receptor tyrosine kinases may display receptor agonistic activity, mimicking the effect of natural ligands. To investigate along this line, we tested the ability of human/mouse equivalent anti-MET antibodies to promote MET auto-phosphorylation in a receptor activation assay. A549 human lung carcinoma cells and MLP29 mouse liver precursor cells were deprived of serum growth factors for 48 hours and then stimulated with increasing concentrations (0-5 nM) of antibodies or recombinant HGF (A549 cells, recombinant human HGF, R&D Systems; MLP29 cells, recombinant mouse HGF, R&D Systems). After 15 minutes of stimulation, cells were washed twice with ice-cold phosphate buffered saline (PBS) and then lysed as described (Longati et al., Oncogene 9, 49-57, 1994). Protein lysates were resolved by electrophoresis and then analysed by Western blotting using antibodies specific for the phosphorylated form of MET (tyrosines 1234-1235), regardless of whether human or mouse (Cell Signaling Technology). The same lysates were also analysed by Western blotting using anti-total human MET antibodies (Invitrogen) or anti-total mouse MET antibodies (R&D Systems). This analysis revealed that all human/mouse equivalent antibodies display MET agonistic activity. Some antibodies promoted MET auto-phosphorylation to an extent comparable to that of HGF (71G3, 71D6, 71C3, 71D4, 71A3, 71G2, 74C8). Some others (76H10, 76G7, 71G12, 72F8) were less potent, and this was particularly evident at the lower antibody concentrations. No clear correlation between MET activation activity and HGF-competition activity was observed.

To obtain more quantitative data, the agonistic activity of antibodies was also characterized by phospho-MET ELISA. To this end, A549 and MLP29 cells were serum-starved as above and then stimulated with increasing concentrations (0-25 nM) of mAbs. Recombinant human (A549) or mouse (MLP29) HGF was used as control. Cells were lysed and phospho-MET levels were determined by ELISA as described (Basilico et al., J Clin Invest. 124, 3172-3186, 2014). Briefly, 96 well-plates were coated with mouse anti-human MET antibodies or rat anti-mouse MET antibodies (both from R&D Systems) and then incubated with cell lysates. After washing, captured proteins were incubated with biotin-conjugated anti-phospho-tyrosine antibodies (Thermo Fisher), and binding was revealed using HRP-conjugated streptavidin (Sigma-Aldrich).

The results of this analysis are consistent with the data obtained by Western blotting. As shown in **Table 14,** 71G3, 71D6, 71C3, 71D4, 71A3, 71G2 and 74C8 potently activated MET, while 76H10, 76G7, 71G12 and 72F8 caused a less pronounced effect. In any case, all antibodies displayed a comparable effect in human and in mouse cells.

Table 14: Agonistic activity of human/mouse equivalent anti-MET antibodies in human and mouse cells as measured by ELISA. A549 human lung carcinoma cells and MLP29 mouse liver precursor cells were serum-starved and then stimulated with increasing concentrations of mAbs. Recombinant human HGF (hHGF; A549) or mouse HGF (mHGF; MLP29) was used as control. Cell lysates were analysed by ELISA using anti-total MET antibodies for capture and anti-phospho-tyrosine antibodies for revealing. Agonistic activity is expressed as EC_{50} (nM) and E_{MAX} (% HGF activity).

mAb	A549 cells		MLP29 cells	
	EC_{50} (nM)	E_{MAX} (%)	EC_{50} (nM)	E_{MAX} (%)
76H10	1.77	61.23	2.91	64.10
71G3	0.41	95.72	0.37	97.81
71D6	0.32	101.57	0.21	114.56
71C3	0.35	86.19	0.33	98.85
71D4	0.59	84.63	0.51	95.34
71A3	0.31	86.56	0.26	95.95
71G2	0.37	101.35	0.25	109.87
76G7	1.86	62.34	1.19	71.45
71G12	2.48	70.61	2.01	75.39
74C8	0.52	87.63	0.41	102.15
72F8	1.51	69.74	0.79	66.82
HGF	0.19	100.00	0.23	100.00

Example 7: Scatter assay

To evaluate whether the agonistic activity of human/mouse equivalent anti-MET antibodies could translate into biological activity, we performed scatter assays with both human and mouse epithelial cells. To this end, HPAF-II human pancreatic adenocarcinoma cells (American Type Culture Collection) and MLP29 mouse liver precursor cells were stimulated with increasing concentrations of recombinant HGF (human or mouse; both from R&D Systems) and cell scattering was determined 24 hours later by microscopy as described previously (Basilico et al., J Clin Invest. 124, 3172-3186, 2014). This preliminary analysis revealed that HGF-induced cell scattering is linear until it reaches saturation at approximately 0.1 nM in both cell lines. Based on these HGF standard curves, we elaborated a scoring system ranging from 0 (total absence of cell scattering in the absence of HGF) to 4 (maximal cell scattering in the presence of 0.1 nM HGF). HPAF-II and MLP29 cells were stimulated with increasing concentrations of human/mouse equivalent anti-MET antibodies, and cell scattering was determined 24 hours later using the scoring system described above. As shown in **Table 15,** this analysis

revealed that all mAbs tested promoted cell scattering in both the human and the mouse cell systems, with substantially overlapping results on both species. 71D6 and 71G2 displayed the very same activity as HGF; 71G3 and 71A3 were just slightly less potent than HGF; 71C3 and 74C8 required a substantially higher concentration in order to match the activity of HGF; 71D4, 76G7, 71G12 and 72F8 did not reach saturation in this assay.

Table 15: Biological activity of human/mouse equivalent anti-MET antibodies as measured in a cell-based scatter assay. HPAF-II human pancreatic adenocarcinoma cells and MLP29 mouse liver precursor cells were stimulated with increasing concentrations of human/mouse equivalent anti-MET antibodies, and cell scattering was determined 24 hours later using the scoring system described in the text (0, absence of cell scattering; 4, maximal cell scattering).

HPAF-II human pancreatic adenocarcinoma cells

mAb	mAb concentration (nM)								
	9.000	3.000	1.000	0.333	0.111	0.037	0.012	0.004	0.001
76H10	3	2	1	0	0	0	0	0	0
71G3	4	4	4	4	3	2	1	0	0
71D6	4	4	4	4	4	3	2	1	0
71C3	4	4	3	2	1	0	0	0	0
71D4	2	2	1	0	0	0	0	0	0
71A3	4	4	4	4	3	3	2	0	0
71G2	4	4	4	4	4	3	2	1	0
76G7	3	2	1	0	0	0	0	0	0
71G12	3	2	2	1	0	0	0	0	0
74C8	4	4	3	3	2	1	0	0	0
72F8	3	2	1	0	0	0	0	0	0
hHGF	4	4	4	4	4	3	2	1	0
IgG1	0	0	0	0	0	0	0	0	0

MLP29 mouse liver precursor cells

mAb	mAb concentration (nM)								
	9.000	3.000	1.000	0.333	0.111	0.037	0.012	0.004	0.001
76H10	3	2	1	0	0	0	0	0	0
71G3	4	4	4	4	2	1	0	0	0
71D6	4	4	4	4	4	3	2	1	0
71C3	4	4	3	2	1	0	0	0	0
71D4	2	2	1	0	0	0	0	0	0
71A3	4	4	4	4	3	3	2	0	0
71G2	4	4	4	4	4	2	1	0	0
76G7	3	2	1	0	0	0	0	0	0
71G12	3	2	2	1	0	0	0	0	0
74C8	4	4	3	3	2	1	0	0	0
72F8	3	2	1	0	0	0	0	0	0
mHGF	4	4	4	4	4	3	2	1	0
IgG1	0	0	0	0	0	0	0	0	0

Example 8: Protection against drug-induced apoptosis

Several lines of experimental evidence indicate that HGF display a potent anti-apoptotic effect on MET-expressing cells (reviewed by Nakamura et al., J Gastroenterol Hepatol. 26 Suppl 1, 188-202, 2011). To test the potential anti-apoptotic activity of human/mouse equivalent anti-MET antibodies, we performed cell-based drug-induced survival assays. MCF10A human mammary epithelial cells (American Type Culture Collection) and MLP29 mouse liver precursor cells were incubated with increasing concentrations of staurosporine (Sigma Aldrich). After 48 hours, cell viability was determined by measuring total ATP concentration using the Cell Titer Glo kit (Promega) with a Victor X4 multilabel plate reader (Perkin Elmer). This preliminary analysis revealed that the drug concentration that induced about 50% cell death is 60 nM for MCF10A cells and 100 nM for MLP29 cells. Next, we incubated MCF10A cells and MLP29 cells with the above determined drug concentrations in the presence of increasing concentrations (0-32 nM) of anti-MET mAbs or recombinant HGF (human or mouse; both from R&D Systems). Cell viability was determined 48 hours later as described above. The results of this analysis, presented in **Table 16,** suggest that human/mouse equivalent antibodies protected human and mouse cells against staurosporine-induced cell death to a comparable extent. While some mAbs displayed a protective activity similar or superior to that of HGF (71G3, 71D6, 71G2), other molecules displayed only partial protection

(76H10, 71C3, 71D4, 71A3, 76G7, 71G12, 74C8, 72F8), either in the human or in the mouse cell system.

Table 16: Biological activity of human/mouse equivalent anti-MET antibodies as measured by a cell-based drug-induced apoptosis assay. MCF10A human mammary epithelial cells and MLP29 mouse liver precursor cells were incubated with a fixed concentration of staurosporine in the the presence of increasing concentrations of anti-MET mAbs or recombinant HGF (human or mouse), and total ATP content was determined 48 hours later. Cell viability was calculated as % total ATP content relative to cells treated with neither staurosporine nor antibodies, and is expressed as EC_{50} and E_{MAX}.

mAb	MCF10A cells		MLP29 cells	
	EC_{50} (nM)	E_{MAX} (%)	EC_{50} (nM)	E_{MAX} (%)
76H10	> 32.00	22.75	> 32.00	27.21
71G3	5.04	65.23	4.85	62.28
71D6	1.48	66.81	0.95	68.33
71C3	31.87	50.16	31.03	51.32
71D4	30.16	51.71	29.84	52.13
71A3	< 0.50	71.70	< 0.50	70.54
71G2	1.06	64.85	1.99	58.29
76G7	25.41	51.93	30.08	50.16
71G12	> 32.00	39.35	> 32.00	39.73
74C8	> 32.00	41.74	> 32.00	37.52
72F8	> 32.00	35.79	> 32.00	43.81
HGF	4.57	59.28	5.35	58.65

Example 9: Branching morphogenesis assay

HGF is a pleiotropic cytokine which promotes the harmonic regulation of independent biological activities, including cell proliferation, motility, invasion, differentiation and survival. The cell-based assay that better recapitulates all of these activities is the branching morphogenesis assay, which replicates the formation of tubular organs and glands during embryogenesis (reviewed by Rosario and Birchmeier, Trends Cell Biol. 13, 328-335, 2003). In this assay, a spheroid of epithelial cells is seeded inside a 3D collagen matrix and is stimulated by HGF to sprout tubules which eventually form branched structures. These branched tubules resemble the hollow structures of epithelial glands, e.g. the mammary gland, in that they display a lumen surrounded by polarized cells. This assay is the most complete HGF assay that can be run in vitro.

In order to test whether human/mouse equivalent anti-MET antibodies displayed agonistic activity in this assay, we seeded LOC human kidney epithelial cells (Michieli et al. Nat Biotechnol. 20, 488-495, 2002) and MLP29 mouse liver precursor cells in a collagen layer as described (Hultberg et al., Cancer Res. 75, 3373-3383, 2015), and then exposed them to increasing concentrations of mAbs or recombinant HGF (human or mouse, both from R&D Systems). Branching morphogenesis was followed over time by microscopy, and colonies were photographed after 5 days. Quantification of branching morphogenesis activity was obtained by counting the number of branches for each spheroid. As shown in **Table 17,** all antibodies tested induced dose-dependent formation of branched tubules. However, consistent with the data obtained in MET auto-phosphorylation assays and cell scattering assays, 71D6, 71A3 and 71G2 displayed the most potent agonistic activity, similar or superior to that of recombinant HGF.

Table 17: Branching morphogenesis assay. Cell spheroids preparations of LOC human kidney epithelial cells or MLP29 mouse liver precursor cells were seeded in a collagen layer and then incubated with increasing concentrations (0, 0.5, 2.5 and 12.5 nM) of mAbs or recombinant HGF (LOC, human HGF; MLP29, mouse HGF). Branching morphogenesis was followed over time by microscopy, and colonies were photographed after 5 days. Branching was quantified by counting the number of branches for each spheroid (primary branches plus secondary branches).

LOC cells

mAb	0 nM	0.5 nM	2.5 nM	12.5 nM
76H10	3.3 ± 1.5	7.3 ± 0.6	11.7 ± 1.5	16.7 ± 1.5
71G3	3.0 ± 1.0	13.7 ± 1.5	19.0 ± 2.6	22.3 ± 2.1
71D6	3.0 ± 1.0	29.0 ± 2.0	29.0 ± 2.6	32.7 ± 1.5
71C3	3.3 ± 0.6	8.7 ± 1.5	12.7 ± 2.1	15.7 ± 2.1
71D4	3.0 ± 1.0	9.0 ± 2.6	15.7 ± 1.2	18.7 ± 1.5
71A3	3.0 ± 1.7	24.0 ± 4.6	30.3 ± 3.2	31.3 ± 1.5
71G2	3.7 ± 1.5	25.3 ± 2.1	29.3 ± 3.5	31.7 ± 3.5
76G7	2.7 ± 0.6	6.7 ± 0.6	13.3 ± 4.2	16.3 ± 5.7
71G12	3.3 ± 0.6	7.0 ± 2.6	15.3 ± 5.5	16.0 ± 4.6
74C8	3.0 ± 1.0	10.3 ± 4.2	17.0 ± 4.6	18.7 ± 4.9
72F8	3.3 ± 1.5	9.0 ± 3.5	12.3 ± 2.1	16.0 ± 3.0
hHGF	3.0 ± 1.0	18.0 ± 2	27.7 ± 2.5	20.3 ± 2.1

MLP29 cells

mAb	0 nM	0.5 nM	2.5 nM	12.5 nM
76H10	0.3 ± 0.6	10.7 ± 4.0	14.3 ± 3.2	24.7 ± 6.0
71G3	0.3 ± 0.6	24.7 ± 4.5	34.3 ± 5.5	29.3 ± 8.0
71D6	1.3 ± 1.2	32.7 ± 3.5	39.0 ± 7.5	41.3 ± 8.0
71C3	0.3 ± 0.6	11.7 ± 3.5	15.7 ± 6.5	24.7 ± 6.5
71D4	0.7 ± 1.2	16.0 ± 2.6	14.7 ± 4.5	21.7 ± 5.5
71A3	0.7 ± 0.6	30.3 ± 2.1	42.0 ± 6.2	42.7 ± 8.0
71G2	1.0 ± 1.0	34.0 ± 2.6	46.3 ± 4.7	45.0 ± 7.0
76G7	0.3 ± 0.6	14.7 ± 2.1	18.7 ± 4.5	24.7 ± 6.5
71G12	1.0 ± 1.0	14.0 ± 2.6	14.7 ± 5.5	22.7 ± 6.0
74C8	0.7 ± 0.6	17.3 ± 2.5	15.3 ± 6.0	22.3 ± 9.0
72F8	1.0 ± 1.0	12.7 ± 3.1	11.7 ± 3.5	18.7 ± 2.5
mHGF	0.7 ± 1.2	32.3 ± 4.0	43.7 ± 4.2	36.0 ± 7.2

Example 10: Fine epitope mapping

In order to finely map the epitopes of MET recognized by human/mouse equivalent anti-MET antibodies we pursued the following strategy. We reasoned that, if an antibody generated in llamas and directed against human MET cross-reacts with mouse MET, then this antibody probably recognizes a residue (or several residues) that is (or are) conserved between *H. sapiens* and *M. musculus* but not among *H. sapiens, M. musculus* and *L. glama.* The same reasoning can be extended to *R. norvegicus* and *M. fascicularis.*

To investigate along this line, we aligned and compared the amino acid sequences of human (UniProtKB # P08581 ; aa 1-932), mouse (UniProtKB # P16056.1 ; aa 1-931), rat (NCBI # NP_1 13705.1 ; aa 1-931), cynomolgus monkey (NCBI # XP_005550635.2; aa 1-948) and llama MET (GenBank # KF042853.1 ; aa 1-931) among each other. With reference to **Table 12,** we concentrated our attention within the regions of MET responsible for binding to the 71D6, 71C3, 71D4, 71A3 and 71G2 antibodies (aa 314-372 of human MET) and to the 76H10 and 71G3 antibodies (aa 546-562 of human MET). Within the former region of human MET (aa 314-372) there are five residues that are conserved in human and mouse MET but not in llama MET (Ala 327, Ser 336, Phe 343, lie 367, Asp 372). Of these, four residues are also conserved in rat and cynomolgus monkey MET (Ala 327, Ser 336, lie 367, Asp 372). Within the latter region of human MET (aa 546-562) there are three residues that are conserved in human and mouse MET but not in llama MET (Arg 547, Ser 553, Thr 555). Of these, two residues are also conserved in rat and cynomolgus monkey MET (Ser 553 and Thr 555).

Using human MET as a template, we mutagenized each of these residues in different permutations, generating a series of MET mutants that are fully human except for specific residues, which are llama. Next, we tested the affinity of selected SEMA-binding mAbs (71D6, 71C3, 71D4, 71A3, 71G2) and PSI-binding mAbs (76H10 and 71G3) for these MET mutants by ELISA. To this end, the various MET proteins were immobilized in solid phase (100 ng/well in a 96-well plate) and then exposed to increasing concentrations of antibodies (0-50 nM) solution. As the antibodies used were in their human constant region format, binding was revealed using HRP-conjugated anti-human Fc secondary antibody (Jackson Immuno Research Laboratories). Wild-type human MET was used as positive control. The results of this analysis are presented in **Table 18.**

Table 18. The epitopes of MET responsible for agonistic antibody binding represent residues conserved among *H. sapiens, M. musculus, R. norvegicus, M. fascicularis* but not among the same species and *L glama.* The relevance of residues conserved among human, mouse, rat, cynomolgus monkey but not llama MET for binding to agonistic mAbs was tested by ELISA. Wild-type (WT) or mutant (MT) human MET ECD was immobilized in solid phase and exposed to increasing concentrations of mAbs in solution. Binding was revealed using anti-human Fc secondary antibodies. All binding values were normalized to the WT protein and are expressed as % binding (E_{MAX}) compared to WT MET.

MT	MUTATIONS	mAb binding (% WT MET ECD)						
		SEMA BINDERS					PSI BINDERS	
		71D6	71C3	71D4	71A3	71G2	76H10	71G3
WT	–	100.0	100.0	100.0	100.0	100.0	–	–
A	1,2,3	103.3	99.8	114.5	116.8	92.1	–	–
B	4,5	0.0	0.0	0.0	0.0	0.0	–	–
C	1,2,3,4,5	0.0	0.0	0.0	0.0	0.0	–	–
D	1,2	128.0	101.8	119.6	127.9	113.5	–	–
E	2,3,4	43.6	59.6	57.2	65.4	41.4	–	–
F	2,4,5	0.0	0.0	0.0	0.0	0.0	–	–
G	3,4,5	0.0	0.0	0.0	0.0	0.0	–	–
H	2,4	38.6	61.6	58.7	76.7	40.2	–	–
I	6,7,8	–	–	–	–	–	100.0	100.0
J	6,7	–	–	–	–	–	89.0	91.2
K	6,8	–	–	–	–	–	0.0	0.0
L	7,8	–	–	–	–	–	0.0	0.0

The results presented above provide a definite and clear picture of the residues relevant for binding to our agonistic antibodies.

All the SEMA binders tested (71D6, 71C3, 71D4, 71A3, 71G2) appear to bind to an epitope that contains 2 key amino acids conserved in human, mouse, cynomolgus and rat MET but not in llama MET lying within blade 5 of the SEMA β-propeller: lie 367 and Asp 372. In fact, mutation of Ala 327, Ser 336 or Phe 343 did not affect binding at all; mutation of lie 367 partially impaired binding; mutation of lie 367 and Asp 372 completely abrogated binding. We conclude that both lie 367 and Asp 372 of human MET are important for binding to the SEMA-directed antibodies tested.

Also the PSI binders tested (76H10, 71G3) appear to bind to a similar or the same epitope. In contrast to the SEMA epitope, however, the PSI epitope contains only one key amino acid also conserved in human, mouse, cynomolgus and rat MET but not in llama MET: Thr 555. In fact, mutation of Arg 547 or Ser 553 did not affect binding at all, while mutation of Thr 555 completely abrogated it. We conclude that Thr 555 represents the crucial determinant for binding to the PSI-directed antibodies tested.

Example 11: Design, generation and characterization of a one-armed MET-specific antagonistic antibody blocking the biological activity of Hepatocyte Growth Factor and cross-reactive with human, mouse, rat and monkey MET

As the results presented so far suggest, all anti-MET antibodies described in this document display agonistic activity, although with different potency. This depends on the ability of the immunoglobulin molecule, which is bivalent, to stabilize the bound antigen (MET) in a dimeric form, leading to receptor trans-phosphorylation and activation. In order to generate a MET antagonistic antibody, also cross-reactive with human, mouse, rat and monkey MET, we transformed a bivalent agonistic antibody selected from the above panel (74C8) into a monovalent, one-armed form (74C8-OA). 74C8-OA consists of a single antigen-binding fragment (Fab) fused to a complete constant domain fragment (Fc) as described before (Merchant et al., Proc Natl Acad Sci. 110:2987-2996, 2013). The one-armed antibody was produced in mammalian cells and gel-purified as described for the other antibodies. The ability of 74C8-OA to bind to human, mouse, rat and monkey MET was assessed by ELISA using a MET ECD in solid phase and increasing concentrations of the antibody in solution. This analysis revealed that 74C8-OA binds with similar affinity to all of these MET proteins. The binding of 74C8-OA to native MET was determined by flow cytometry on human and mouse epithelial cells expressing MET. Cells were incubated with increasing concentrations of the antibody, and binding was revealed by flow cytometry analysis. The results obtained in these experiments indicated that 74C8-

OA binds to native MET on the surface of living cells. In order to assess the ability of 74C8-OA to displace human or mouse HGF, an HGF competition assay was performed by ELISA. MET-Fc protein, either human or mouse, was immobilized in solid phase and then exposed to biotinylated human or mouse HGF in the presence of increasing concentrations of the antibody. HGF binding was measured by horse radish-conjugated streptavidin. This analysis revealed that 74C8-OA is a potent displacer of HGF in both human and mouse HGF/MET systems.

Both the agonistic and antagonistic activity of 74C8-OA were characterized by phospho-MET ELISA on mouse and human epithelial cells. For the agonistic activity assay, serum-starved cells were stimulated with increasing concentrations of 74C8-OA, lysed and then adsorbed on goat anti-human MET antibody (R&D Systems) in solid phase. Phospho-MET was revealed using a rabbit anti-pMET (Y1234-Y1235) antibody (Cell Signaling) and a secondary HRP-conjugated goat anti-rabbit antibody (Pierce). This analysis revealed that the agonistic activity of 74C8-OA is negligible at all concentrations tested. For the antagonistic activity, serum-starved mouse and human epithelial cells were stimulated with a fixed concentration (100 ng/ml) of recombinant human HGF (R&D Systems) in the presence of increasing concentrations of 74C8-OA. MET activation was determined by phospho-MET ELISA as described above. This analysis demonstrated that 74C8-OA displays strong MET antagonistic activity by inhibiting HGF-induced MET auto-phosphorylation.

We conclude that the 74C8-OA antibody is a potent MET antagonistic antibody that, in contrast to the MET agonist antibodies provided herein, does not display any significant MET agonistic activity.

Example 12: The 71D6 MET agonistic antibody inhibits chronic inflammation-induced colorectal fibrosis and carcinogenesis

To cast light onto the potential pro-tumorigenic effect of MET activation in a chronic colon inflammation setting, we compared the pharmacological effect of a MET agonistic antibody (71D6) with that of a MET antagonistic antibody (74C8-OA) in a classic two-hit colon carcinogenesis mouse model. To this end, we exposed 8 week-old female BALB/c mice (Charles River) to a single i.p. injection with azoxymethane (AOM; a potent mutagen for epithelial cells of the gastro-intestinal tract) at a dose of 12.5 mg/kg followed by three cycles of dextran sodium sulphate (DSS; a potent inducer of colon inflammation and ulceration) dissolved in the drinking water at a concentration of 6% (weight/volume). Each inflammatory cycle consisted of 7 days of DSS administration followed by 14 days on regular water. On day 8, when the first cycle of DSS started, mice were randomized into 4

arms of 11 mice each which received respectively: (i) vehicle only (PBS); (ii) the MET agonistic 71D6 antibody at a dose of 1 mg/kg ; (iii) the MET agonistic 71D6 antibody at a dose of 5 mg/kg; (iv) the MET antagonistic antibody 74C8-OA at a dose of 5 mg/kg. An additional, fifth control arm contained 7 mice that received no AOM-DSS or antibody and served as healthy control. Mice were sacrificed 16 days after the third DSS cycle was interrupted. At autopsy, colons were collected, washed through, and their length and weight were determined. Following measurement, colons were cut open longitudinally and stained with 1% Alcian Blue solution to highlight tumour masses. Tumours were counted and photographed under a stereo-microscope. At the end of this procedure, colons were fixed in 4% paraformaldehyde, embedded in paraffin and processed for histological analysis. During the whole course of the experiment, mouse weight was monitored on a regular basis, and the clinical symptoms of ulcerative colitis were assessed by determining fecal blood, rectal bleeding and stool consistency. Quantification was achieved using a standard scoring system used in pre-clinical models (Kim et al., J Vis Exp. 60, pii: 3678, 2012): each parameter scored from 0 (absence of the symptom) to 3 (maximal manifestation of the symptom). Scores relative to the single parameters were summed together to give rise to the Disease Activity Index (DAI) ranging from 0 to 9.

As shown in Figure 1A, exposure to DSS caused a weight loss that increased at each cycle (cycle 1, up to 15%; cycle 2, up to 20%; cycle 3, up to 25%). The DAI increased to a score of 3 or higher during cycle 1 and significantly worsened during cycles 2 and 3, reaching values higher than 4 during the last cycle (Figure 1B). Remarkably, animals fail to recover after the last cycle and continued to display a DAI of 3 or higher, indicating that the inflammatory state could not be reverted at this stage. Consistent with the idea that HGF promotes colonic mucosa integrity and inhibits inflammation, the 71D6 agonistic antibody reduced DSS-induced weight loss and accelerated recover, as well as dramatically inhibited the clinical symptoms of colon inflammation throughout the experiment as measured by DAI analysis. The 74C8-OA antagonistic antibody did not have any effect on either body weight or DAI until cycle 2 but, consistent with the idea that endogenous HGF may act as a natural factor against tissue injury, appeared to worsen the clinical signs of colon inflammation at cycle 3 and after.

As determined at autopsy, DSS reduced colon length by 30% (Figure 2A), while it increased colon specific weight (expressed as grams/cm) up to 93% (Figure 2B). Notably, 71D6 treatment both at 1 and 5 mg/kg prevented colon shortage limiting it to non-significant variations, and maintained colon specific weight values very close to those of healthy control mice. On the contrary, the 74C8-OA MET antagonistic antibody performed similar to vehicle alone both in terms of colon length and colon specific weight.

Following length and weight measurement, colons were opened with a longitudinal cut and stained with 1% Alcian Blue solution, as described above. Colon specimens were analyzed by placing the flattened tissue under a stereo-microscope with their inner (lumen) side towards the lens, and photographed. This analysis revealed that AOM/DSS treatment resulted in the induction of a plethora of polyps at the level of the mid-colon, at approximately half way between the cecum joint and the anus (Figure 3). Surprisingly, the agonistic 71D6 antibody dramatically reduced colorectal carcinogenesis in this model, while the antagonistic 74C8-OA antibody did not affect AOM/DSS-induced tumour formation at all. Tumour number was assessed by counting the number of polyps that were protruding from the otherwise flat mucosa over the entire colon. All mice exposed to AOM/DSS displayed some tumour in the colon. However, remarkably, the 71D6 agonistic antibody reduced the number of tumour masses by more than 50% (Figure 4A). Notably, this reduction was statistically significant ($p < 0.05$) compared to the vehicle alone arm both at 1 and 5 mg/kg, thus confirming the efficacy of the treatment even at the lower dose tested. On the contrary, the antagonistic anti-MET 74C8-OA antibody did not significantly reduce the number of tumours, thus implying that MET agonistic activity and not just MET binding is essential for inhibiting colon carcinogenesis in the presence of persistent inflammation. Colon images were analysed using Image J software (National Institutes of Health) and the volume of the polyps was calculated using the formula $V = \frac{3}{4}\pi (X/2) \cdot (Y/2)^2$, where V is the volume of the polyp, and X and Y are the major and minor dimensions of the polyp section, respectively (in mm). As shown in Figure 4B, 71D6 reduced not only the number but also the size of tumour masses in a dose-dependent manner (1 mg/kg 50% reduction; 5 mg/kg, 25% reduction) compared to the vehicle alone arm. Even more strikingly, 71D6 was very effective in decreasing total tumour burden, reaching 77% reduction at the 1 mg/kg dose and 89% at the 5 mg/kg dose (Figure 4C). Not less importantly, treatment with the antagonistic anti-MET 74C8-OA antibody did not affect any of the tumour parameters measured, i.e. mean polyp number, mean tumour volume and total tumour burden.

Following tissue processing and paraffin embedding, colon specimens were cut using a microtome and prepared for histological and immunohistochemical analysis. First, sections were stained with hematoxylin and eosin and examined by microscopy. This analysis confirmed that AOM/DSS treatment caused chronic inflammation of the colonic mucosa leading to the development of large malignant lesions (Figure 5). Histological analysis of tumours revealed that all lesions represent high grade adenomas of the colonic epithelium (also known as in situ carcinoma of the colon). Strikingly, the 71D6 antibody at both doses almost completely suppressed adenoma formation, maintaining a

normal morphology of the mucosa, substantially indistinguishable from the control group. The antagonistic 74C8-OA antibody, on the contrary, did not affect AOM/DSS-induced tumour formation at all.

Next, we determined whether chronic colon inflammation resulted in fibrosis. To this end, colon sections were stained by various techniques specific for the detection of fibrotic tissue, including the Piero Sirius red method, which highlights collagen, and anti-alpha smooth muscle actin (α-SMA) antibodies, which specifically stain myofibroblasts. These analyses revealed that repeated DSS administration caused the insurgence of massive fibrosis in colonic tissue. Collagen-rich fibrotic tissue is particularly evident where tumour masses are present (Figure 6). Remarkably, colon sections derived from animals treated with both AOM-DSS and 71D6 showed a significant lower collagen deposition and milder fibrosis at 1 mg/kg as well as at the higher dose without any evident difference between the two. Colon sections derived from 74C8-OA-treated animals displayed an extent of collagen deposition and fibrosis comparable to that of the vehicle alone arm. A similar pattern of the distribution was observed with α-SMA staining, with higher presence of myofibroblasts in the vehicle alone control arm as well as in the 74C8-OA arm, compared to mice that received 71D6 at either doses (Figure 7).

Colon sections were also stained for the expression of transforming growth factor beta (TGF-β). TGF-β signalling has been demonstrated to be frequently deregulated in human cancers, including colorectal cancer (Massague, Cell 134:251-230, 2008; Xu et al., Hum Mol Genet. 16(SPEC):R14-R20, 2009). While in normal or premalignant cells it usually acts as a tumour suppressor, in advanced cancer it is frequently overexpressed and the growth inhibitory function switch to an oncogenic one thus promoting tumour cell proliferation and invasion (Nagaraj et al., Expert Opin Investig Drugs 19:77-91, 2010). Staining of colon sections with anti-TGF-β antibodies revealed that TGF-β expression is increased by the AOM-DSS treatment and particularly in tumour tissue (Figure 8). Notably, treatment with the 71D6 agonistic antibody restored TGF-β levels comparable to those observed in control healthy mice both at 1 and at 5 mg/kg. In contrast, colon sections from mice treated with the 74C8-OA antagonistic antibody displayed a TGF-β expression indistinguishable from that of the vehicle alone arm.

These data suggest that MET activation and not blockade is beneficial in chronic inflammatory pathologies of the intestine, and that administration of a MET-activating drug can both reduce the clinical signs of chronic colon inflammation (such as weight loss, diarrhoea, rectal bleeding, blood in stool, colon thickening, collagen deposition, myofibroblast proliferation and fibrosis) and suppress the development of chronic inflammation-induced colorectal cancer. We suggest that the 71D6 antibody or other

similar MET agonistic antibodies may be used in the clinic to treat pathological conditions associated with chronic colon inflammation, including colitis-associated fibrosis and especially cancer.

5   Example 13: The 7 1D6 MET agonistic antibody inhibits DNA-damaaina aaent-induced colorectal carcinogenesis

The results obtained in the AOM/DSS model suggest that MET activation using an agonistic antibody dramatically reduces the risk of developing colorectal cancer, typically associated with chronic inflammation of the intestine. However, colorectal cancer may 10   also arise from gene mutations in the epithelial cells of the colonic mucosa that accumulate during long periods of time. The particular anatomical site of these epithelial cells expose them to a number of agents introduced with the food or drink that may cause DNA mutations, particularly if food is contaminated with pollutants. Furthermore, pathological conditions of the intestine such as disbiosys or altered permeability can 15   enhance the occurrence of gene mutations in the colonic mucosa.

In order to determine whether MET activation via an agonistic antibody can affect mutagenesis-promoted colorectal carcinogenesis, we tested the 7 1D6 molecule in a the following setting. We injected 7 week-old female BALB/c mice (Charles River) with the colon-specific mutagen AOM at a dose of 5 mg/kg once a week for 6 weeks. Starting from 20   day 1, mice were randomized into 2 arms of 2 1 mice each which received treatment with 7 1D6 (at a dose of 5 mg/kg) or vehicle only (PBS). Antibody was administered two times a week by i.p. injection. An additional, third control arm contained 7 mice that received no AOM or antibody and served as healthy control. Mice were sacrificed 8 weeks after the last AOM injection, i.e. 14 weeks after the experiment started. At autopsy, colons were 25   collected and washed through. Explanted colons were measured using a ruler and weighed. Following measurements, colons were cut open longitudinally to expose tumour masses. Tissues were stained ex vivo with a 1% Alcian Blue solution in order to highlight tumour borders. Polyps were counted and photographed under a stereo-microscope. At the end of this procedure, colons were fixed in 4% paraformaldehyde, embedded in 30   paraffin and processed for histological analysis.

In contrast to AOM/DSS, and consistent with the absence of inflammation, AOM alone did not affect colon length (Figure 9A) or specific weight (Figure 9B) compared to control mice. 7 1D6 treatment also did not change significantly either parameter. However, AOM-induced mutagenesis did result in the development of colorectal cancer, although to 35   a reduced extent compared to AOM/DSS in terms of both incidence (Figure 10A) and number (Figure 10B). This is consistent with the idea that chronic inflammation boosts

tumour progression in many organs, including the intestine. Interestingly, treatment with the 71D6 agonistic antibody significantly inhibited AOM-promoted cancer: in fact, both tumour incidence (Figure 10A) and number (Figure 10B) decreased by approximately 60% in the arm receiving 71D6.

5   Figure 11 shows two representative images of explanted colons for each arm. Colon samples were stained using a 1% Alcian Blue solution. The arrows indicate macroscopically evident tumour masses. Colon sections were then processed for histology. This analysis indicated that the polips observed under the stereo-microscope are low grade adenomas, therefore less malignant than those observed in the AOM/DSS
10   model.

      Example 14: Reduction of colonic inflammation by MET agonist antibodies

      We tested whether agonistic anti-MET antibodies could reduce intestinal inflammation in a mouse model, since intestinal (colorectal) inflammation is a major risk
15   factor in developing colorectal cancer. To this end, we exposed 7 week-old female BALB/c mice (Charles River) to dextran sodium sulphate (DSS) in the drinking water for 10 days. On day 10, DSS treatment was interrupted and mice were put back on normal water. Starting from day 1, mice were randomized into 7 arms of 7 mice each which received treatment with 71G3, 71D6, 71G2 (at a dose of 1 mg/kg or 5 mg/kg) or vehicle only (PBS).
20   Antibodies were administered three times a week by i.p. injection. An additional, eighth control arm contained 7 mice that received no DSS or antibody and served as healthy control. Mice were sacrificed on day 12, i.e. 2 days after DSS administration was interrupted. At autopsy, colons were collected, washed through, and their length was determined using a ruler. Following measurement, colons were embedded in paraffin and
25   processed for histological analysis.

      During the whole course of the experiment, mouse weight was monitored on a regular basis, and the clinical symptoms of intestinal inflammation were assessed by determining faecal blood, rectal bleeding and stool consistency. Quantification was achieved using a standard scoring system used in pre-clinical models (Kim et al., J Vis
30   Exp. 60, pii: 3678, 2012): each parameter scored from 0 (absence of the symptom) to 3 (maximal manifestation of the symptom). Scores relative to the single parameters were summed together to give rise to the Disease Activity Index (DAI) ranging from 0 to 9.

      As shown in Figure 12, exposure to DSS in the PBS arm caused a weight loss of up to 25%; the DAI increased to a score of 4 or higher; and the length of the colon was
35   reduced by up to 40%. Remarkably, all antibodies analyzed reversed these effects in a dose-dependent fashion, displaying significant activity already at the lower dose tested.

7 1D6 was the most potent antibody: after a transient decline, it brought body weight back at normal values, comparable to those observed in the PBS group; it curbed the DAI increase, substantially inhibiting all the clinical symptoms; and it prevented colon shortage, limiting it to negligible variations.

5   Colon sections were stained with hematoxylin and eosin and examined by microscopy. As shown in Figure 13, DSS administration caused profound damage to the colonic mucosa. The epithelial layer appeared eroded and infiltrated with lymphocytes. The colonic mucosa was disseminated with cryptic abscess sites and was heavily colonized by foamy macrophages, responsible for tissue destruction. Peri-visceral lymph
10   nodes appeared enlarged. The muciparous glands were characterized by atrophy and displayed marked mucinous depletion, which was substituted with inflammatory infiltrate including foamy macrophages, lymphocytes and neutrophils. Several ulcers were visibly invaded by granulocytic or macrophage exudate, leading to the total disappearance of the glandular component. Remarkably, mice treated with both DSS and agonistic anti-MET
15   antibodies displayed much milder symptoms of degeneration and inflammation. Specifically, elements of acute inflammation were absent, including macrophages and granulocytes; the mucosa appeared only marginally injured, displaying sparse glandular distortion and rarefaction; mucin secretion was restored, and erosions and ulcers were completely absent. Although these protective effects were dose-dependent in all antibody
20   groups, they were already evident at 1 mg/kg, indicating that the concentrations of antibodies reached with this dose are very close to saturation. In this model as well, the most effective antibody appeared to be 7 1D6, though all tested agonist antibodies were effective.

25   <u>Conclusion</u>

We conclude that treatment with an HGF-MET agonist (in this case a MET agonistic antibody) can be beneficial in both the treatment of colorectal cancer associated with chronic inflammation, and also in the inhibition of tumours arising from mutations in the colonic tissue. Agonist antibody 7 1D6 has been demonstrated to be particularly
30   effective in treating these conditions. Other MET agonist antibodies described herein (for example 7 1G3 and 7 1G2) also exhibit a potent ability to reduce intestinal inflammation comparable to 7 1D6 and will therefore provide similar therapeutic and preventative effects.

Moreover, the results herein demonstrate that MET agonists are more effective at
35   treating (colorectal) cancer than the native MET ligand HGF. Yamaji *et at. Oncology Reports* 26: 335-341, 201 1 (incorporated herein by reference) describe administering

HGF to mice in similar models to those described herein. However, HGF is not as effective as MET agonists such as anti-MET agonist antibodies - for example, HGF only reduces tumour incidence in AOM-treated mice from 65% to about 30% (Yamaji *et at,* Table I), compared to MET agonist antibodies which reduce tumour incidence to less than

5   20% (Figure 10).

We suggest that treatment with MET agonists, particularly MET agonist antibodies such as 71D6, may be used in the clinic for reducing the development of colorectal tumours in those individuals predisposed to colorectal cancer, for example in patients affected by familial adenomatous polyposis (FAP; characterized by *APC* or *MUTYH* gene

10   mutations) or by other genetic syndromes, as well as those predisposed to colorectal cancer as a result of inflammatory bowel conditions.

Claims

1.   A method of treating cancer comprising administering to a subject in need thereof an HGF-MET agonist.

2.   The method of claim 1, wherein the cancer is colorectal cancer.

3.   The method of claim 1 or claim 2, wherein the subject has been identified as at increased risk of colorectal cancer.

4.   A method of treating colorectal fibrosis, comprising administering to a subject an HGF-MET agonist.

5.   The method of any one of claims 1-4, wherein the subject has been diagnosed with colorectal inflammation prior to administration of the HGF-MET agonist.

6.   The method of any one of claims 1-5, wherein the subject has inflammatory bowel disease, optionally ulcerative colitis or Crohn's disease.

7.   The method of any one of claims 1-6, wherein the subject has a family history of familial adenomatous polyposis (FAP).

8.   The method of any one of claims 1-7, wherein the HGF-MET agonist is administered at a dose in the range from 0.1-10 mg/kg per dose.

9.   The method of any one of claims 1-8, wherein the HGF-MET agonist is administered at a dose in the range from 0.5-10 mg/kg, optionally from 1-5 mg/kg.

10.   The method of any one of claims 1-9 wherein the HGF-MET agonist is administered at a dose of 1 mg/kg or 5 mg/kg.

11.   The method according to any one of claims 1-10, wherein the HGF-MET agonist is administered at least once per week, optionally 1-3 times per week, optionally twice per week.

12.   The method according to any one of claims 1-11, wherein the treatment is prophylactic treatment.

13.   The method according to any one of claims 1-11, wherein the treatment is therapeutic treatment.

14.   An HGF-MET agonist for use in a method according to any one of claims 1-13.

15.   A pharmaceutical composition for use in a method according to any one of claims 1-13, wherein the pharmaceutical composition comprises an HGF-MET agonist and a pharmaceutically acceptable excipient or carrier.

16.   A method, HGF-MET agonist for use or pharmaceutical composition for use according to any preceding claim, wherein the HGF-MET agonist is a full agonist of MET.

17.   A method, HGF-MET agonist for use or pharmaceutical composition for use according to any preceding claim wherein, wherein the HGF-MET agonist is an anti-MET agonist antibody or antigen-binding fragment thereof.

18.   A method, antibody for use or pharmaceutical composition for use according to claim 17 wherein the anti-MET antibody or antigen-binding fragment thereof binds to the SEMA domain of MET, optionally blades 4-5 of the SEMA β-propeller.

19.   A method, antibody for use or pharmaceutical composition for use according to claim 17 or claim 18, wherein the anti-MET antibody or antigen-binding fragment thereof binds to an epitope comprising residue Ile367 and/or Asp372 of MET, optionally comprising both residue Ile367 and Asp372 of MET.

20.   A method, antibody for use or pharmaceutical composition for use according to claim 17 wherein the anti-MET antibody or antigen-binding fragment thereof binds to the PSI domain of MET, optionally binds an epitope between residues 546 and 562 of MET.

21.   A method, antibody for use or pharmaceutical composition for use according to claim 17 or claim 20, wherein the anti-MET antibody or antigen-binding fragment thereof binds to an epitope comprising residue Thr555 of MET.

22.   A method, antibody for use or pharmaceutical composition for use according to any one of claims 17-19, wherein the anti-MET agonist antibody or antigen-binding fragment comprises the combination of VH CDR1, CDR2 and CDR3 sequences and VL CDR1, CDR2 and CDR3 sequences of 71D6 or of 71G2.

23.   A method, antibody for use or pharmaceutical composition for use according to claim 22 wherein the anti-MET agonist antibody or antigen-binding fragment comprises a VH domain at least 90% identical to SEQ ID No 163 and/or comprises a VL domain at least 90% identical to SEQ ID No 164.

24.   A method, antibody for use or pharmaceutical composition for use according to any one of claims 17-23 wherein the anti-MET agonist antibody is an IgG4 antibody.

25.   A method, antibody for use or pharmaceutical composition for use according to any one of claims 17-24 wherein the anti-MET agonist antibody is 71D6.

26.   A method of treating colorectal cancer in a subject, comprising administering to the subject an effective amount of anti-MET antibody 71D6.
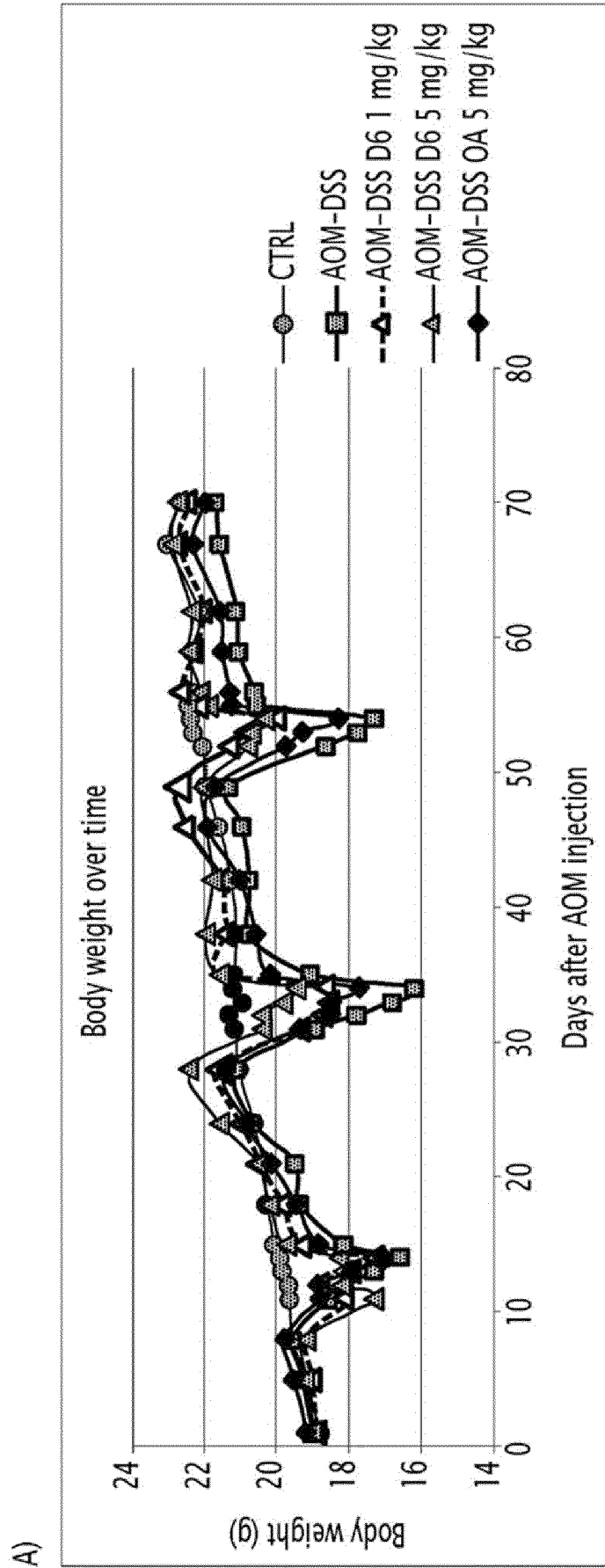
FIG.1 beginning
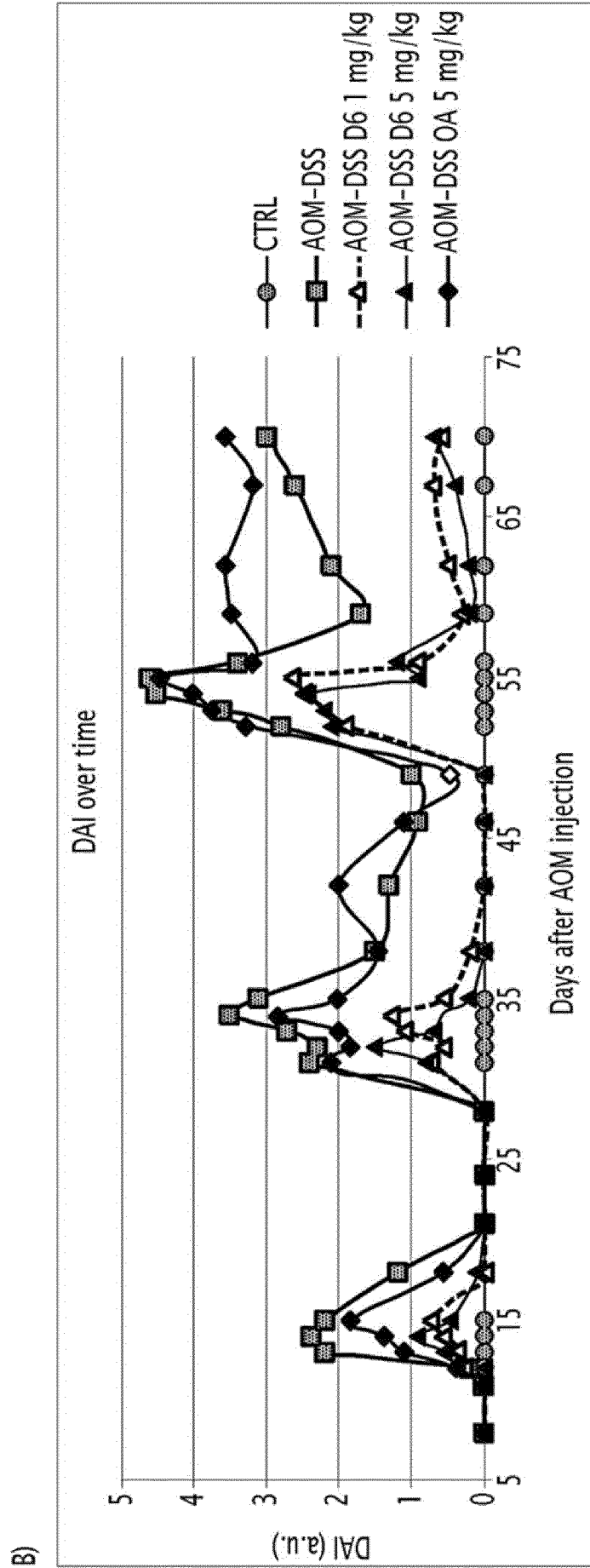
FIG.1 end
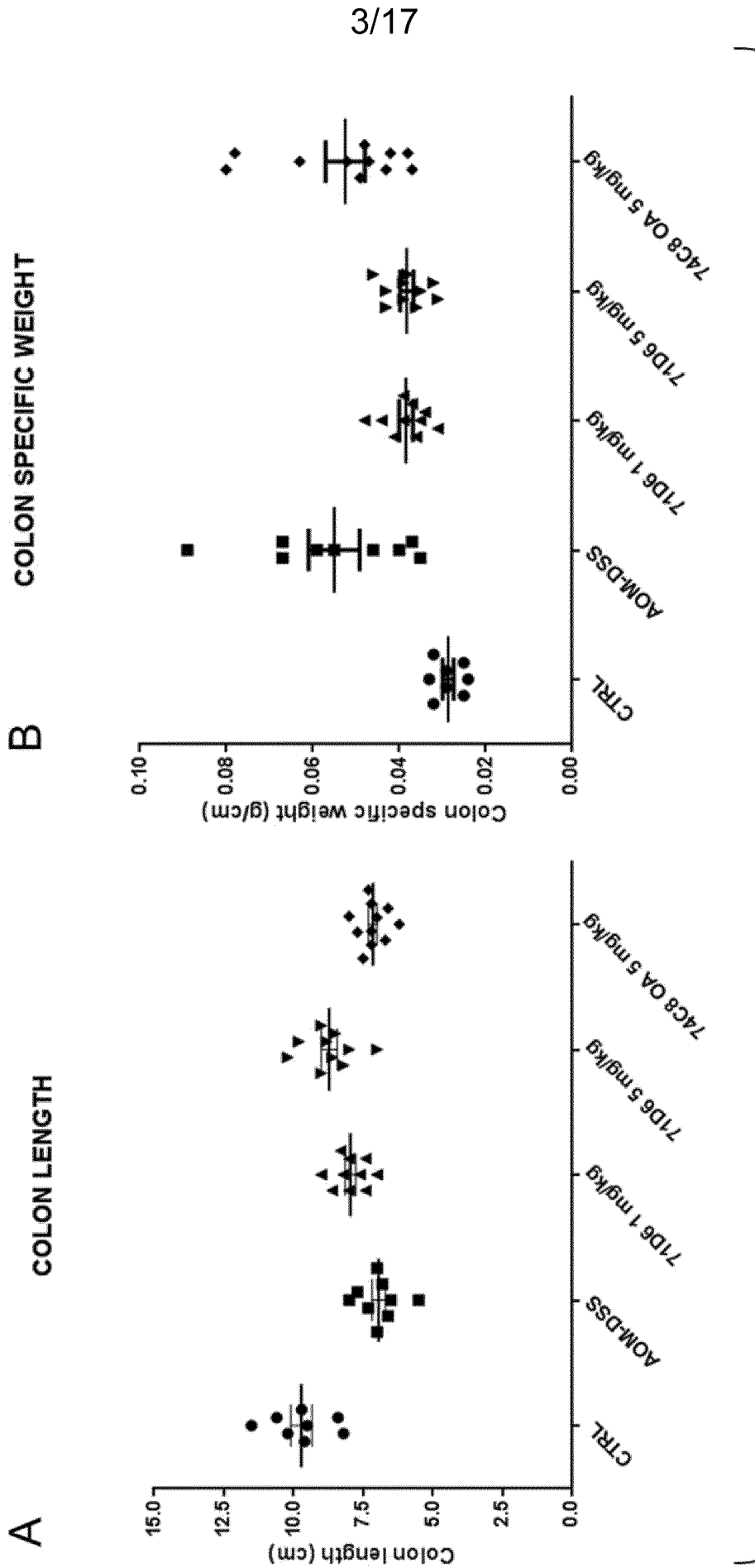
FIG.2

Macroscopic colon examination: tumors highlighted by Alcian blue staining

AOM/DSS

AOM/DSS + 5 mg/kg 74C8

AOM/DSS

AOM/DSS + 5 mg/kg 71D6

Normal colon

AOM/DSS + 1 mg/kg 71D6

FIG.3

FIG.4 beginning

TOTAL TUMOR BURDEN

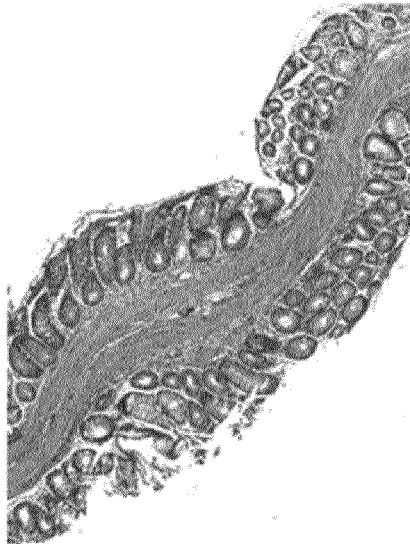FIG.4 end

H&E (100X): colon morphology and presence of tumors

FIG.5

Picro-sirius red (400X): staining for collagen

AOM/DSS

AOM/DSS + 5 mg/kg 71C8

AOM/DSS
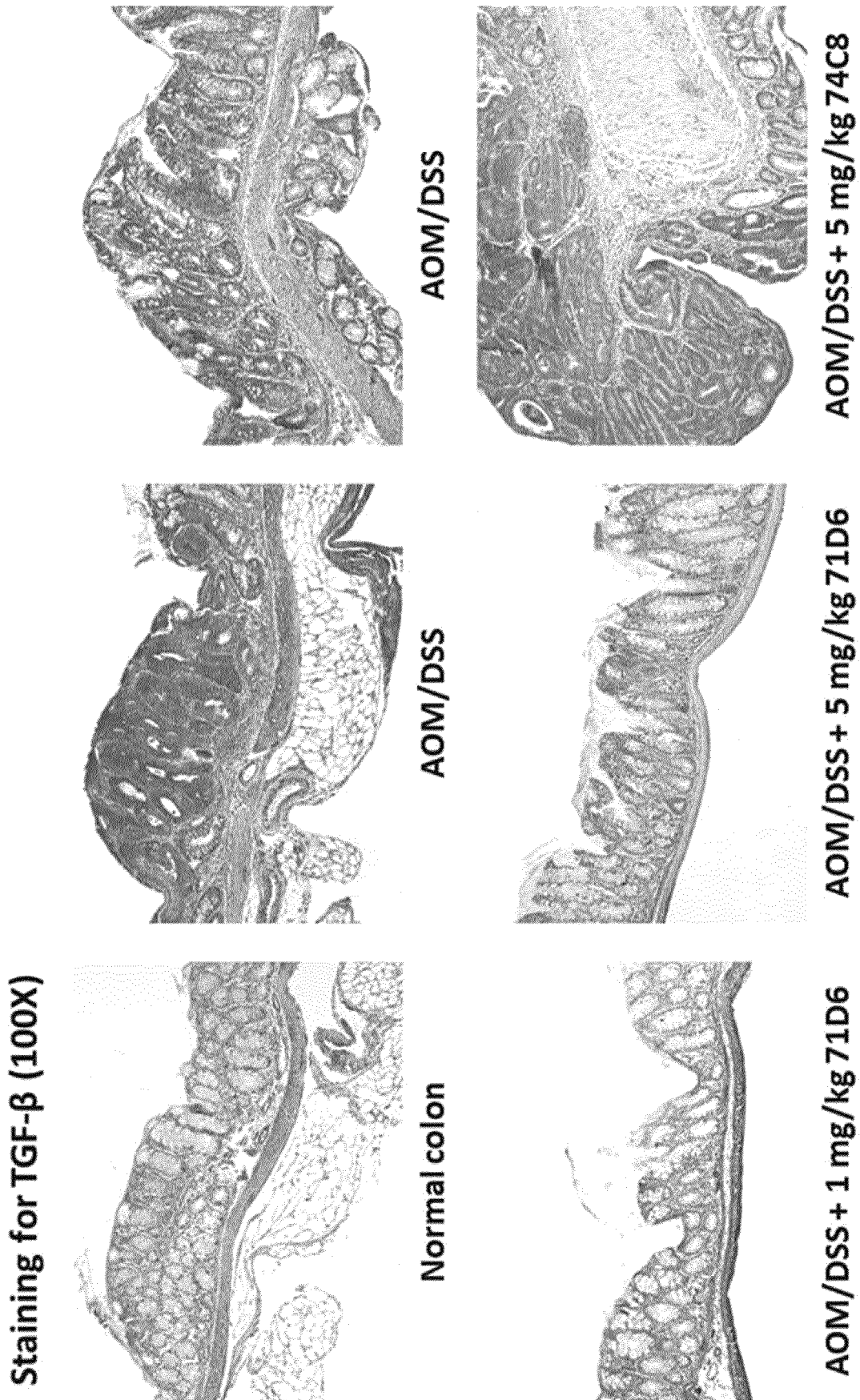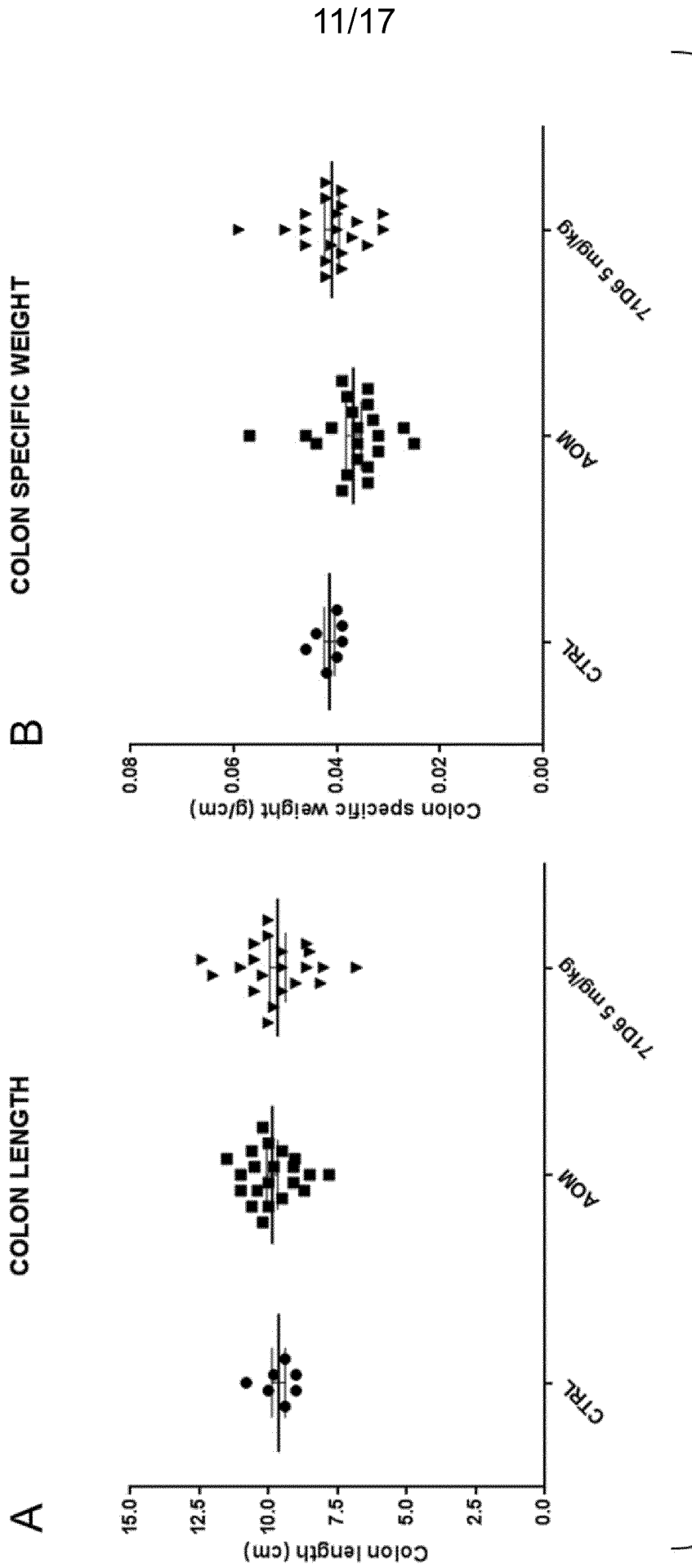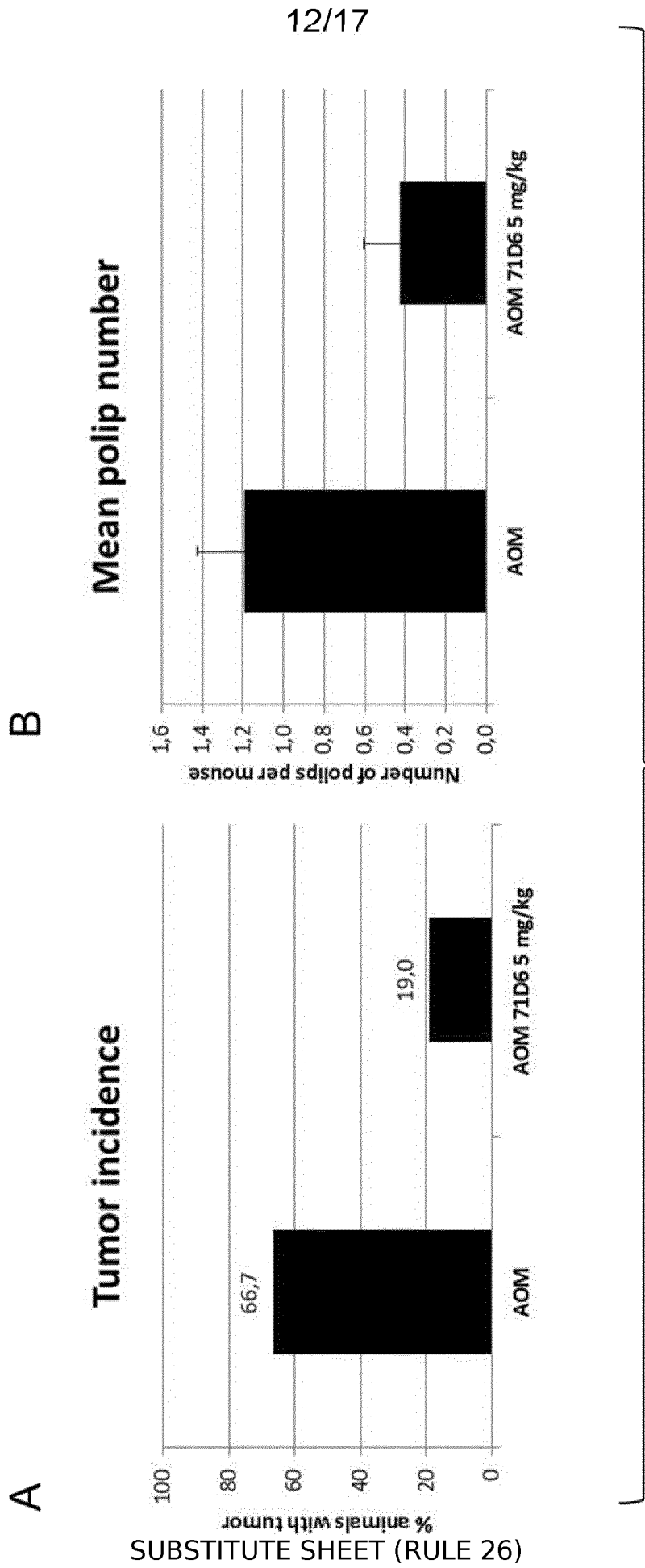
AOM/DSS + 5 mg/kg 71D6

Normal colon

AOM/DSS + 1 mg/kg 71D6

FIG.6

α-SMA (400X): staining for myofibroblasts
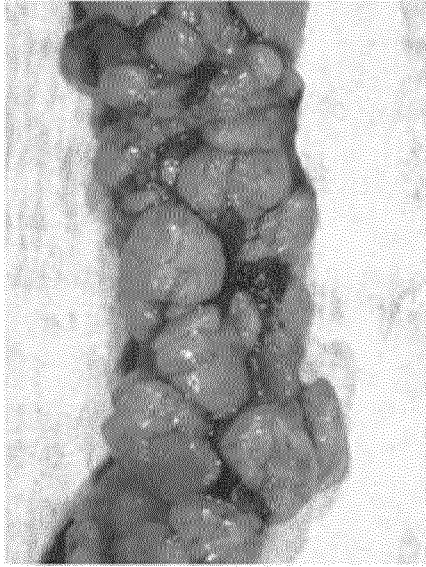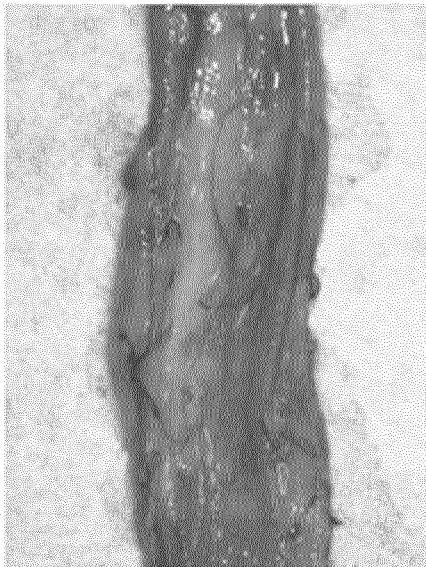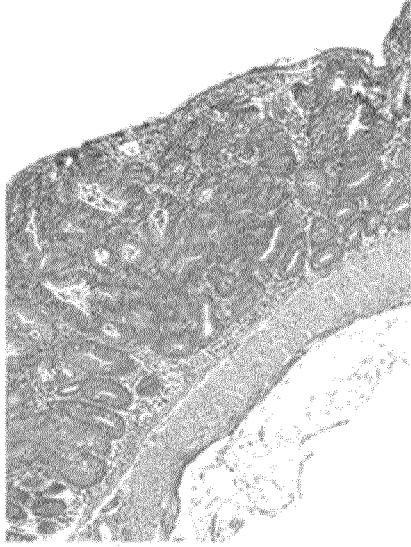
AOM/DSS
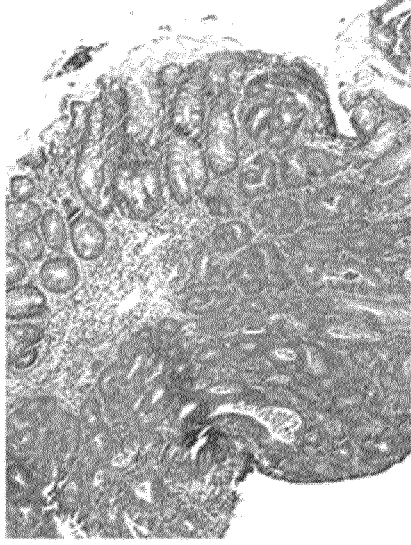
AOM/DSS

Normal colon
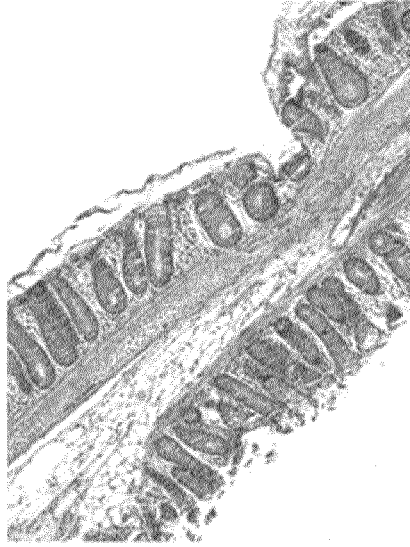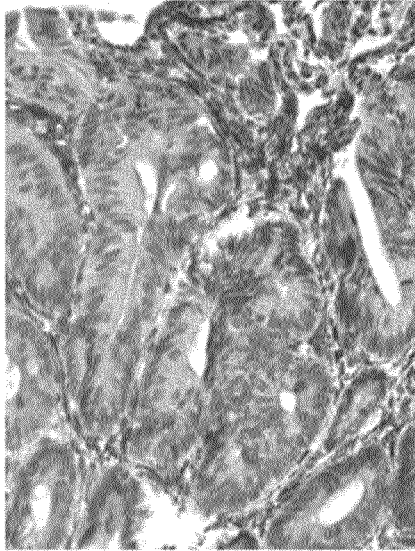
AOM/DSS + 5 mg/kg 74C8
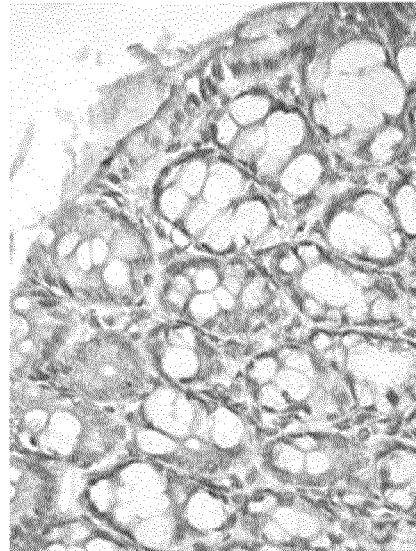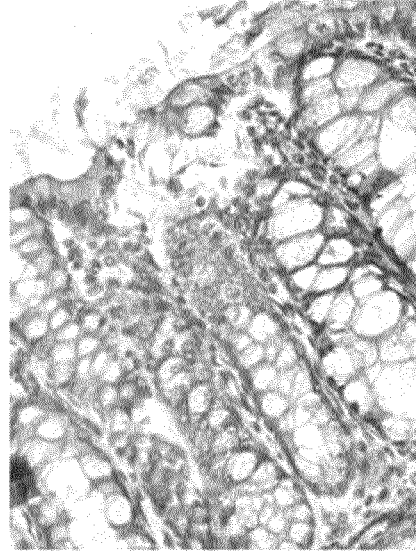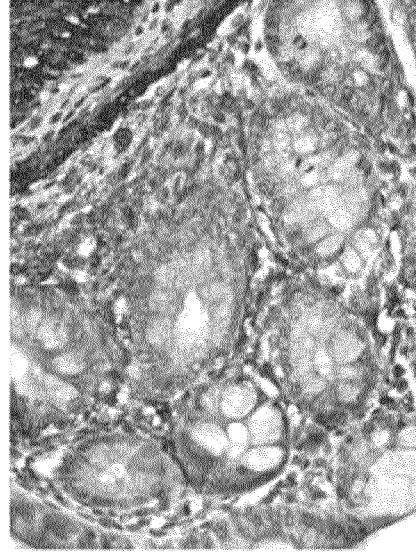
AOM/DSS + 5 mg/kg 71D6

AOM/DSS + 1 mg/kg 71D6

FIG.7

Staining for TGF-β (100X)

AOM/DSS

AOM/DSS + 5 mg/kg 74C8

AOM/DSS

AOM/DSS + 5 mg/kg 71D6

Normal colon

AOM/DSS + 1 mg/kg 71D6

FIG.8

FIG.9

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FIG.10

Macroscopic colon examination: tumors highlighted by Alcian blue staining

FIG.11

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FIG.12 beginning

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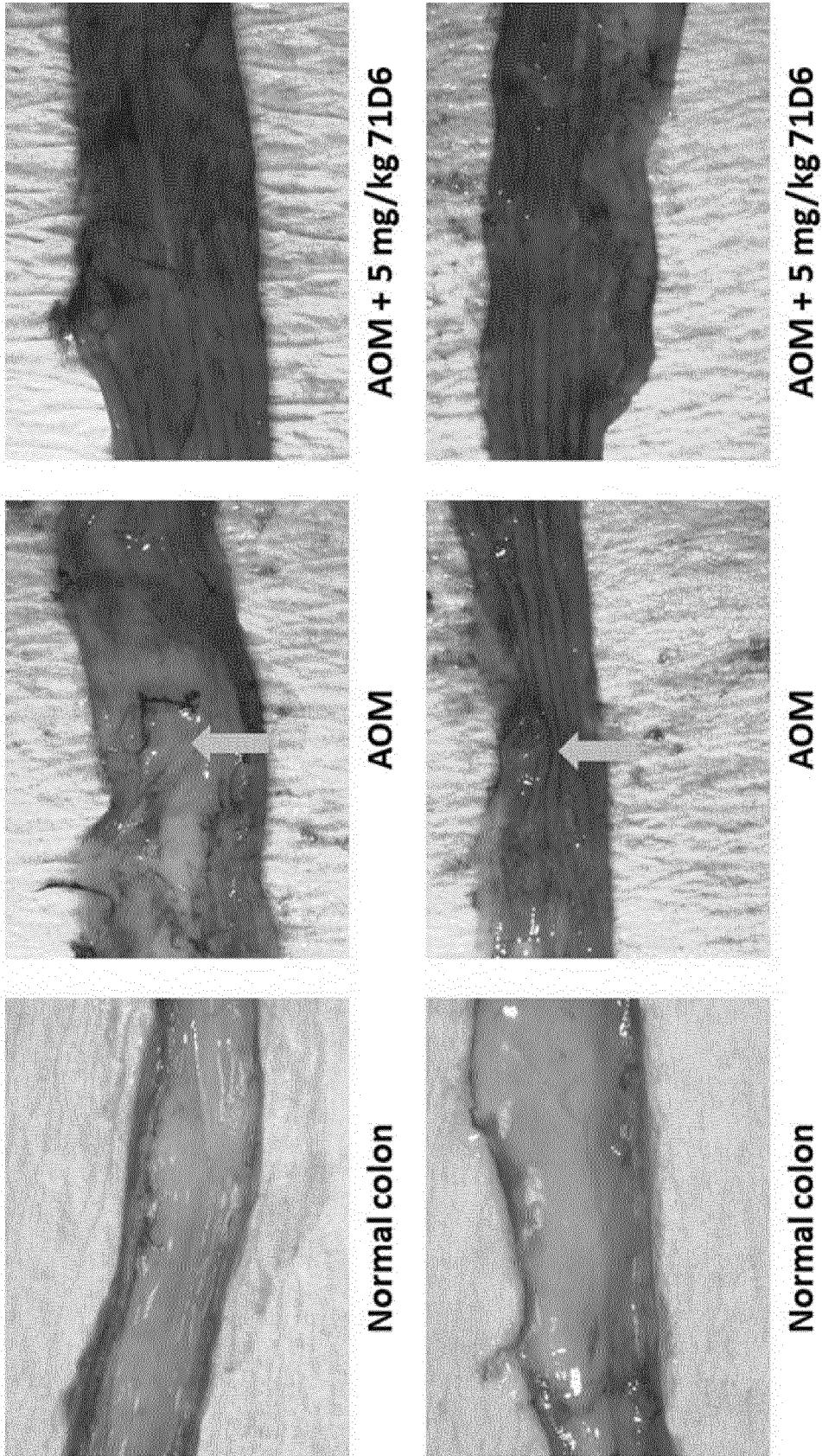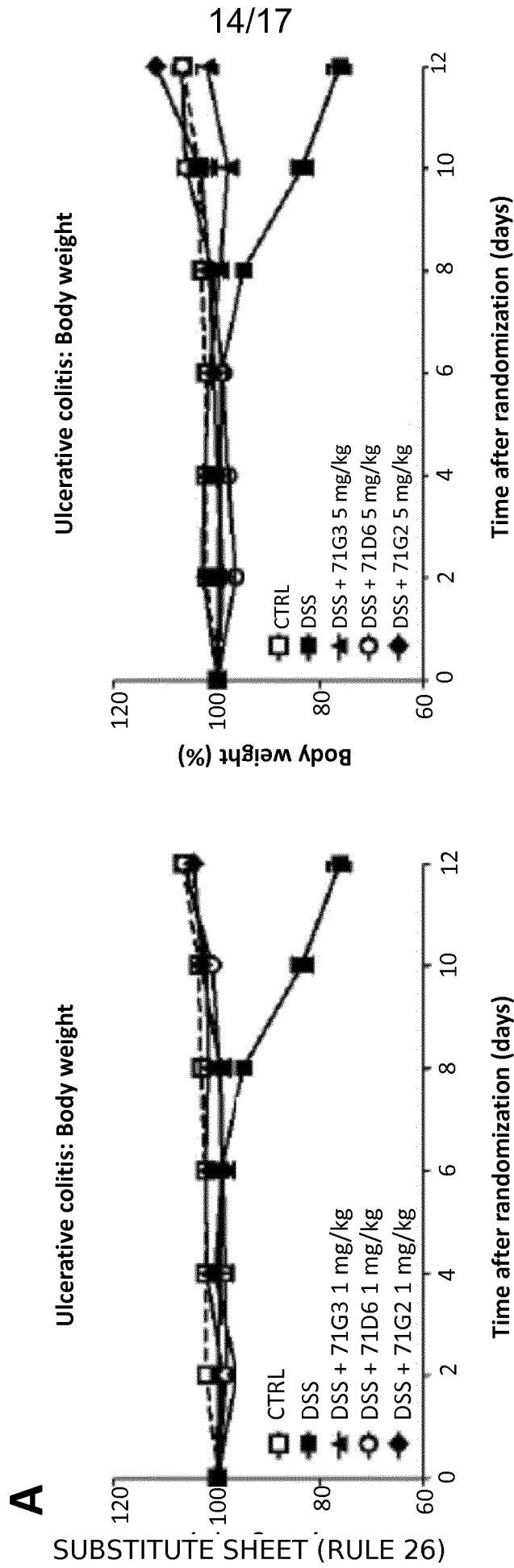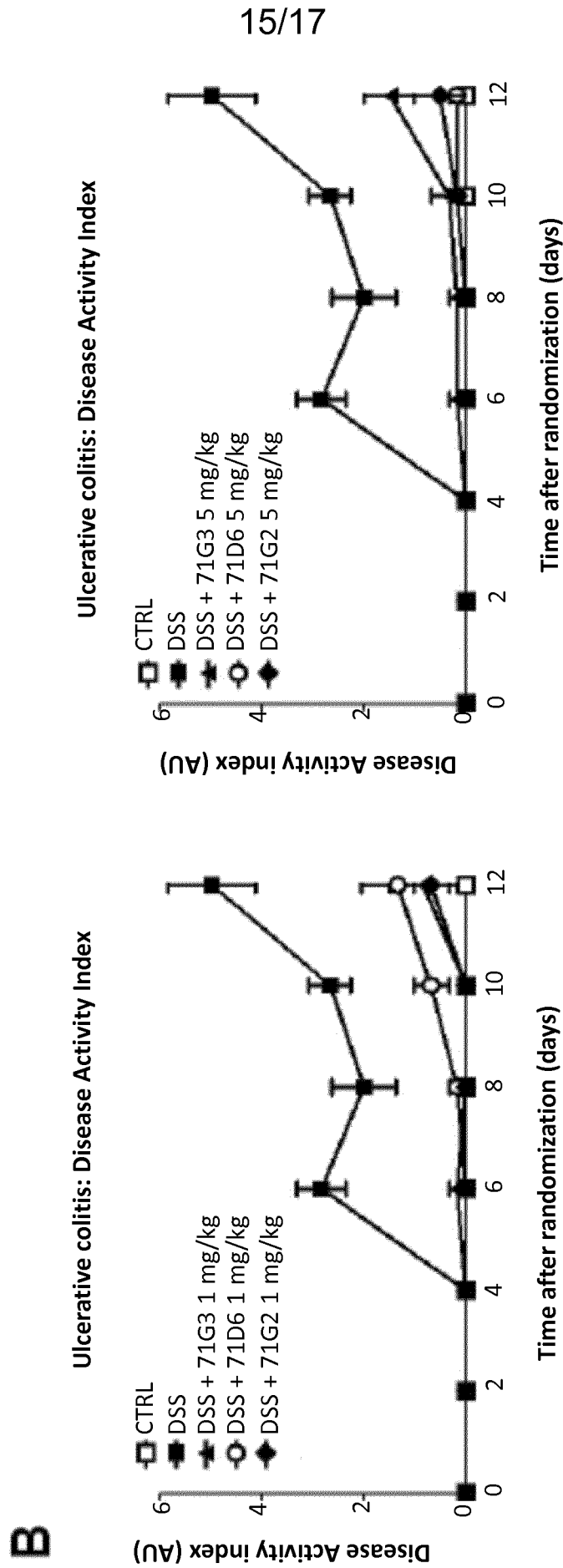
FIG.12 continued

FIG.12 end

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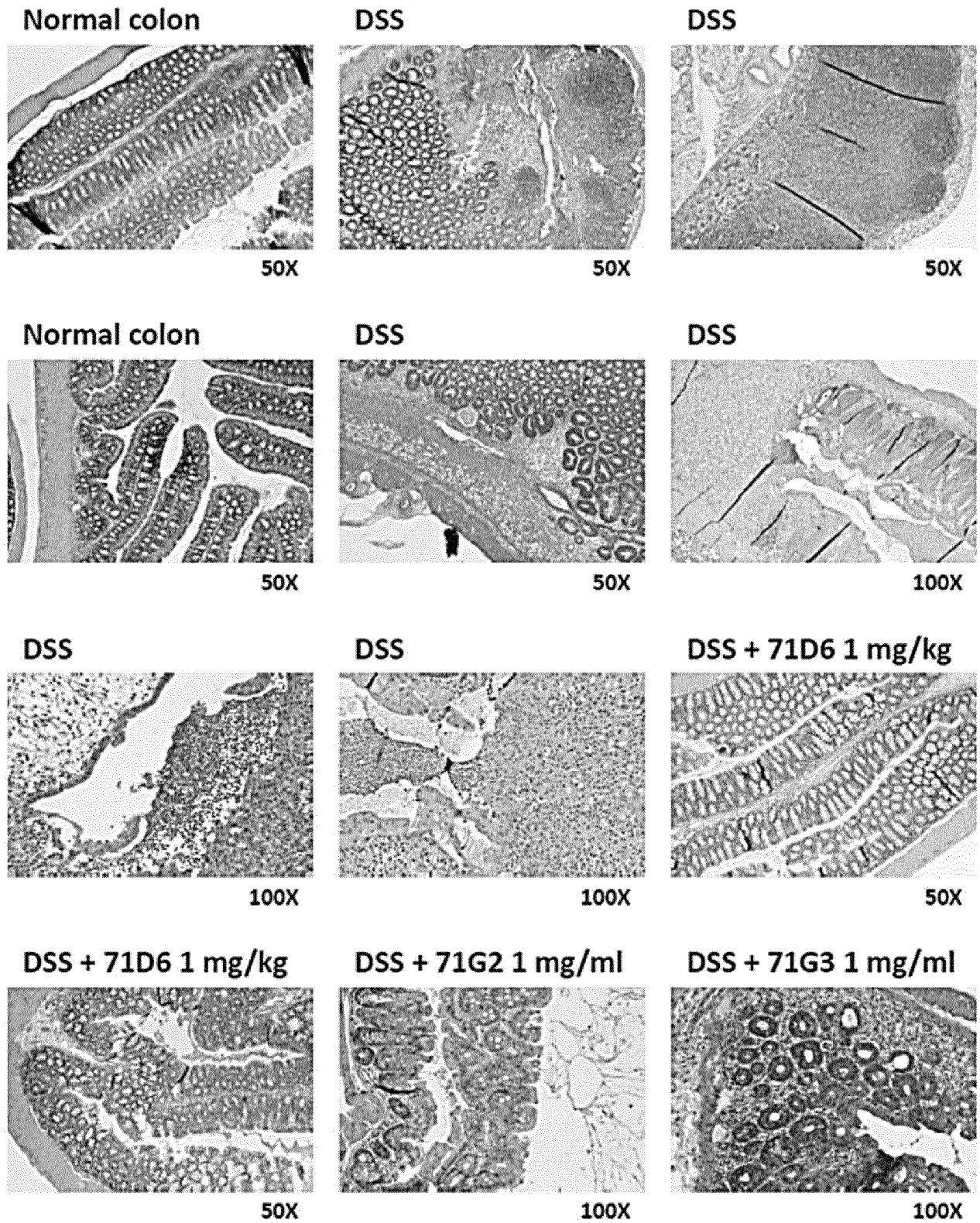
FIG.13

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28   A61K39/395   A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K   A61K   A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*' '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 2016/106221 A1 (UNIV ROCKEFELLER [US]) 30 June 2016 (2016-06-30) exampl es 7.5, 7.8 f i gures 9, 12 -----	1, 12-26
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X	Further documents are listed in the continuation of Box C.		X	See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2019	25/03/2019

Name and mailing address of the ISA/	Authorized officer
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C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

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C(Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

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