Silenced antibody-based anti-MET constructs for the treatment of tumors and metastasis

Filed of the invention

The present disclosure relates to novel silenced antibody-based therapeutic agent for the treatment of tumors and/or metastasis. The therapeutic agents of the present disclosure are monovalent and are specific for MET.

Background of the invention

Cancer is a genetic disease in which somatic endogenous genes undergo mutations. Only a handful of genes – known as oncogenes and tumor suppression genes – are altered in cancer cells and drive tumorigenesis. The activated oncogenes are accelerators and the inactivated tumor suppressor genes are missing brakes for cancer cell growth. Subsequent to the discovery of cancer-causing mutated genes, a new concept emerged for oncogenes: the "oncogene addiction", which indicates that a cancer cell, despite its abundance of genetic alterations, is still dependent on a single oncogenic protein for its sustained proliferation or survival. Therefore, new therapeutic inroads were undertaken to develop "target therapy" to cure cancer. In the last 20 years, pharmacological targeting of proteins encoded by cancer-causing mutated genes – through chemical drugs or antibodies - has represented the forefront armamentarium to eliminate the mutated cells and combat cancer disease. Target therapies promise to be more effective than conventional cytotoxic chemotherapies, often with fewer side effects. However, only the patients bearing in their tumors the altered specific target gene are likely to benefit from target therapy. Thus, personalized medicine is required in parallel to assess the druggable genetic lesions through comprehensive genomic profiling of the patient tumor.

The MET oncogene encodes for a unique receptor tyrosine kinase endowed with pleiotropic functions. When genetically altered (by point mutations, gene fusion, translocation, and/or amplification), MET initiates transformation of the cells by virtue of its ability to activate the invasive growth program. Thus, MET genetic lesions leading to constitutive Met kinase hyperactivation initiate and maintain the transformed phenotype ("MET addiction").

MET genetic lesions occur in most solid tumors with an overall frequency of 1-4% and are able to upregulate its kinase activity1. Point mutations are concentrated in domains critical for Hepatocyte Growth Factor (HGF) ligand binding or receptor signalling (SEMA domain, juxtamembrane domain, and catalytic domain). Most recently, next-generation sequencing revealed exon 14 splice site mutations in 3% of non-small cell lung cancers2, which lead to exon skipping and deletion of the juxtamembrane region of the MET transcript, where a serine residue (Ser985) negatively regulates the Met kinase activity3 and a tyrosine residue (Tyr1003) is required for Met internalization and degradation4.

In the "invasive growth" program elicited by MET, the proliferative response is coupled with migration, survival, extracellular matrix degradation, and induction of cell polarity5. These biological responses are strived by cells to adapt to adverse conditions and/or escape to find a more convenient environment. In a hostile context, Met is overexpressed – via transcriptional upregulation – by a variety of stimuli such as hypoxia, inflammatory cytokines, pro-angiogenic factors, mitogens and even HGF itself. Lastly, Met is overexpressed in conditions of radiation-induced DNA damage and contributes to resistance to radiotherapy by promoting activation of DNA repair and evasion of programmed cell death of cancer cells.

Several Met-targeting molecules have been developed to erase the hyperactive Met signalling in a selective, robust, and highly effective manner. These drugs include: HGF antagonists (either blocking antibodies or decoys), mAbs targeting the Met receptor, and chemical tyrosine kinase inhibitors (TKIs). Anti-Met mAbs potentially represent a major step in the battle against the cancers driven by MET. Nowadays, four anti-Met mAbs have entered early clinical trials: MetMab (Onartuzumab, Roche), LY2875358 (Emibetuzumab, Eli Lilly & Company), ARGX-111 (Argenx), SAIT301 (Samsung) and Sym015 (Symphogen A/S), a mixture of two antibodies. They act by blocking HGF binding to MET in a competitive fashion (Onartuzumab, ARGX-111) and/or downregulating MET (Emibetuzumab, SAIT301, Sym015).

The murine DN30 mAb (disclosed in WO 2007/090807) is an IgG2A which binds the extracellular domain of the human Met receptor and induces only some of the Met-triggered biological effects6. It partially activates receptor phosphorylation due to its bivalent nature which allows simultaneous binding to two distinct antigen molecules, resulting in stabilization of receptor complexes in a fashion similar to that achieved by natural ligands. This unwanted partial agonistic activity on Met was not observed in the monovalent DN30 Fab fragment (MvDN30)7. Conversion of the bivalent DN30 parental antibody into the monovalent Fab fragment unleashes the therapeutic potential of the DN30 anti-Met antibody, leading to a full antagonist molecule. However, the short half-life of the Fab, due to its low

molecular weight, is a severe limitation for the deployment in therapy. The present inventors thus developed new engineered molecules called DCD (Dual Constant Domain Fab) characterized by the duplication of the constant domains present in the DN30 Fab: DCD-1, in which the duplication was done in tandem, and DCD-2, in which the constant domains of the light and heavy chain were reciprocally swapped (disclosed in WO 2014/108829). Both the new recombinant molecules show biochemical properties in vitro comparable to the original Fab, acting as full Met antagonists. In vivo, upon systemic administration, the new recombinant molecules reduce Met-addicted tumor growth. DCD-1 and DCD-2 show a pharmacokinetic profile improved over the original DN30 Fab, nevertheless none of two reach the behavior comparable to the mAb of origin8.

WO2020/074459 discloses monovalent agents specific for Met. In these agents, one arm of the antibody was deleted by molecular engineering, leading to an improved in vivo stability which is attributable to the activity of the Fc domain, which binds the Fc receptor expressed in the organs. One of these monovalent agents, hOA-DN30, is further described in J Exp Clin Cancer Res (2022) Mar 29;41(1): 112. A different one-armed anti-c-Met antibody for the treatment of glioblastoma is disclosed in Clin Cancer Res (2006) 12, 6144. In the present disclosure such agents were further improved by introducing silencing mutation in the Fc region. In addition to the effects commonly attributed to such mutations, such mutations also have important additional safety aspects. The abrogation of Fc gamma receptor binding reduces the residual risk of cMET dimerization through immune cell Fc gamma receptor mediated crosslinking on the tumor cell surface.

Summary of the invention

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide

comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain.

In certain embodiments, said humanized VL domain is fused to the human CL domain in the N- to C-terminal direction. In certain embodiments, the humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction. In certain embodiments, the human hinge region is fused to the CH2 domain that is fused to the human CH3 domain in the N- to C-terminal direction, wherein the human hinge region is truncated at the N-terminus.

In certain embodiments, said humanized VL domain has an amino acid sequence as set forth in SEQ ID No.: 13. In certain embodiments said humanized VH domain has an amino acid sequence as set forth in SEQ ID No.: 14. In certain embodiments, said human CL domain is a human light kappa type domain. In certain embodiments, said human hinge region and the human constant domains CH1, CH2 and CH3 are from a human IgG1. In certain embodiments, the two Fc polypeptides are linked through intermolecular disulfide bonds at the hinge region.

In certain embodiments, the first Fc polypeptide and the second Fc polypeptide meet at an interface, and one between the first and the second Fc polypeptide comprises a knob at the interface, and the other between the first and the second Fc polypeptide comprises a hole at the interface, wherein the knob is positionable into the hole. In certain embodiments, either the first or the second Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries an amino acid mutation at position 389, wherein the original amino acid at position 389 has been mutated to import an amino acid having a larger side chain volume than the original amino acid; and wherein the other Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries three amino acid mutations at positions 389, 391 and 438, wherein the original amino acids have been mutated to import amino acids having smaller side chains volume than the original amino acids, wherein the amino acid numbering is according to the EU numbering scheme of Kabat. In certain embodiments, the original amino acids at positions 389, 391 and 438 are threonine, leucine and tyrosine respectively; and wherein in the first or the second Fc

polypeptide the threonine in position 389 has been mutated to tryptophan; and wherein in the other Fc polypeptide the threonine at position 389 has been mutated to serine, the leucine at position 391 has been mutated to alanine and the tyrosine at position 438 has been mutated to valine.

In certain embodiments, the human CL domain has an amino acid sequence as set forth in SEQ ID No.: 15 and the human CH1 domain has an amino acid sequence as set forth in SEQ ID No.: 16. In certain embodiments, the first human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 17, and the second human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 18.

In certain embodiments, the anti-Met antibody fragment of the present disclosure induces shedding of an extracellular domain of Met when bound to Met.

In certain embodiments, the Fc region of the first Fc polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A (according to EU index), or the mutations L234A, L235E, G237A, A330S and P331S (according to EU index). In certain embodiments, first FC polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A(according to EU index).

In certain embodiments the first polypeptide comprises the amino acid sequence of SEQ ID No. 19, the second polypeptide comprises the amino acid sequence of SEQ ID No. 20, and the third polypeptide comprises the amino acid sequence of SEQ ID No. 18.

In certain embodiments, the present disclosure relates to an isolated nucleic acid encoding any of aforementioned anti-Met antibody fragments. In certain embodiments, the present disclosure relates to a composition comprising two or more recombinant nucleic acids which collectively encode aforementioned anti-Met antibody fragments.

In certain embodiments, the present disclosure relates to any of the aforementioned anti-Met antibody fragments for use in the treatment of a tumor and/or metastasis. In certain embodiments, the present disclosure relates to any of the aforementioned anti-Met antibody fragments for use in the treatment of a tumor and/or metastasis in a patient carrying genetic alterations of the MET gene. In certain embodiments, the present disclosure relates to any of the aforementioned anti-Met antibody fragments for use in the treatment of a tumor and/or metastasis in a patient carrying a wild-type MET gene.

In certain embodiments, the present disclosure relates to a process for the manufacturing of an of aforementioned anti-Met antibody fragments, the process comprising the following steps: (i) synthesis of cDNA sequences of the first, second and third polypeptides constituting the anti-Met antibody fragment, (ii) insertion of the three cDNA sequences into one or more plasmids, wherein the plasmid(s) is(are) suitable for expression in a mammalian cell line, (iii) transient or stable co-transfection of a mammalian cell line with the plasmid(s), (iv) collection of the culture supernatant, (v) purification by affinity chromatography of the anti-Met antibody fragment.

Figure legends

Figure 1 shows a SCX chromatograms of the formats tested. All three formats showed a similar charged species distribution. VERT-004 appears to be slightly more acidic.

Figure 2 shows a RP chromatogram of the formats tested. VERT-004 shows a somewhat different profile compared to VERT-001 and VERT-002 indicated by the presence of multi-peaks.

Figure 3 shows a mass analysis of each individual peak present in VERT-001, VERT-002 and VERT-004 of the RP chromatogram.

Figure 4 shows the binding of VERT001, VERT002 and VERT004 to the ECD of c-Met was measured in an ELISA assay.

Figure 5 shows the anti-proliferative activity of VERT-001, VERT-002 and VERT-against Hs746T cells (left) and EBC-1 cells (right).

Figure 6 shows the monomer, the HMW and the LMW content of VERT-001, VERT-002 and VERT-004 as determined by SEC analysis. RM= reference material fresh aliquot of VERT001).

Figure 7 shows the formation of charge variants as analyzed via strong cation exchange chromatography. RM= reference material fresh aliquot of VERT001).

Figure 8 shows the binding of VERT001, VERT002 and VERT004 to the ECD of c-Met after incubation at 5°C and 37°C for 4 weeks compared to freshly thawed reference material.

Figure 9 shows the analysis of VERT-002 stressed at pH3. An SEC chromatogram is shown on the left, a SCX chromatogram on the right.

Figure 10 shows the analysis of the anti-proliferative activity of the stressed samples of VERT001, VERT002 and VERT004.

Figure 11: Female hairless SCID mice were implanted s.c. with Hs746T tumor cells on Day 0 and treated with isotype control, VERT-001, VERT-002 or VERT-004 for 28 days. Tumor volume (mm3) data are displayed as mean +/- standard error of the mean (SEM); 9 mice were included in each group.

Figure 12: Female hairless SCID mice were implanted s.c. with Hs746T tumor cells on Day 0 and treated with isotype control or VERT-002 antibody. Tumor volume (mm3) data are displayed as mean +/- standard error of the mean (SEM); 9 mice were included in each group. A) Dose response on tumor growth inhibition; B) tumor growth in vivo on Day 35.

Figure 13: Female nude Balb/c mice were implanted s.c. with EBC-1 tumor cells and were given chronic treatment for 28 days with isotype control or VERT-002. Tumor volume (mm3) is displayed as mean and SEM; 10 mice were treated per group. A) Dose-response on tumor growth inhibition with chronic dosing on Days 1, 4, 8, 11, 15, 18, 22, and 25 after randomization; B) Tumor growth in vivo on Day 28; C) Levels of plasma soluble MET ectodomain (sMET ECD) in response to the various treatment conditions, as indicated.

Definitions

The disclosure pertains to antibodies and antibody-based constructs which specifically bind to Met, and the uses of such constructs, in particular therapeutic uses, such as the treatment of tumors and metastasis.

The terms "**Met**", "**cMET**", "**cMet**" and "**MET**", refer to a protein also known as Hepatocyte Growth Factor Receptor, HGFR, or c-Met. Human Met has the following amino acid sequence (UniProt P08581):

MKAPAVLAPGILVLLFTLVQRSNGECKEALAKSEMNVNMKYQLPNFTAETPIQNVILHEH
HIFLGATNYIYVLNEEDLQKVAEYKTGPVLEHPDCFPCQDCSSKANLSGGVWKDNINMAL
VVDTYYDDQLISCGSVNRGTCQRHVFPHNHTADIQSEVHCIFSPQIEEPSQCPDCVVSAL
GAKVLSSVKDRFINFFVGNTINSSYFPDHPLHSISVRRLKETKDGFMFLTDQSYIDVLPE
FRDSYPIKYVHAFESNNFIYFLTVQRETLDAQTFHTRIIRFCSINSGLHSYMEMPLECIL
TEKRKKRSTKKEVFNILQAAYVSKPGAQLARQIGASLNDDILFGVFAQSKPDSAEPMDRS
AMCAFPIKYVNDFFNKIVNKNNVRCLQHFYGPNHEHCFNRTLLRNSSGCEARRDEYRTEF

TTALQRVDLFMGQFSEVLLTSISTFIKGDLTIANLGTSEGRFMQVVVSRSGPSTPHVNFL
LDSHPVSPEVIVEHTLNQNGYTLVITGKKITKIPLNGLGCRHFQSCSQCLSAPPFVQCGW
CHDKCVRSEECLSGTWTQQICLPAIYKVFPNSAPLEGGTRLTICGWDFGFRRNNKFDLKK
TRVLLGNESCTLTLSESTMNTLKCTVGPAMNKHFNMSIIISNGHGTTQYSTFSYVDPVIT
SISPKYGPMAGGTLLTLTGNYLNSGNSRHISIGGKTCTLKSVSNSILECYTPAQTISTEF
AVKLKIDLANRETSIFSYREDPIVYEIHPTKSFISGGSTITGVGKNLNSVSVPRMVINVH
EAGRNFTVACQHRSNSEIICCTTPSLQQLNLQLPLKTKAFFMLDGILSKYFDLIYVHNPV
FKPFEKPVMISMGNENVLEIKGNDIDPEAVKGEVLKVGNKSCENIHLHSEAVLCTVPNDL
LKLNSELNIEWKQAISSTVLGKVIVQPDQNFTGLIAGVVSISTALLLLLGFFLWLKKRKQ
IKDLGSELVRYDARVHTPHLDRLVSARSVSPTTEMVSNESVDYRATFPEDQFPNSSQNGS
CRQVQYPLTDMSPILTSGDSDISSPLLQNTVHIDLSALNPELVQAVQHVVIGPSSLIVHF
NEVIGRGHFGCVYHGTLLDNDGKKIHCAVKSLNRITDIGEVSQFLTEGIIMKDFSHPNVL
SLLGICLRSEGSPLVVLPYMKHGDLRNFIRNETHNPTVKDLIGFGLQVAKGMKYLASKKF
VHRDLAARNCMLDEKFTVKVADFGLARDMYDKEYYSVHNKTGAKLPVKWMALESLQTQKF
TTKSDVWSFGVLLWELMTRGAPPYPDVNTFDITVYLLQGRRLLQPEYCPDPLYEVMLKCW
HPKAEMRPSFSELVSRISAIFSTFIGEHYVHVNATYVNVKCVAPYPSLLSSEDNADDEVD
TRPASFWETS (SEQ ID No. 1)

The term "**antibody**" as used herein refers to a protein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, which interacts with an antigen. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FR's arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The term "antibody" includes for example, monoclonal antibodies, human antibodies, humanized antibodies, camelised antibodies and chimeric antibodies. The antibodies can be of any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., Igd , IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass. Both the light and heavy chains are divided into regions of structural and functional homology.

The term "**antibody fragment**", as used herein, refers to one or more portions of an antibody that retain the ability to specifically interact with (e.g., by binding, steric hindrance, stabilizing spatial distribution) an

antigen. Examples of binding fragments include, but are not limited to, a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward et al., (1989) Nature 341 :544-546), which consists of a VH domain; and an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al., (1988) Science 242:423-426; and Huston et al., (1988) Proc. Natl. Acad. Sci. 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antibody fragment". These antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antibody fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, (2005) Nature Biotechnology 23:1 126-1 136). Antibody fragments can be grafted into scaffolds based on polypeptides such as Fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies). Antibody fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1 -VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen-binding sites (Zapata et al., (1995) Protein Eng. 8: 1057-1062; and U.S. Pat. No. 5,641 ,870).

The structures and locations of immunoglobulin variable domains, e.g., CDRs, may be defined using well known numbering schemes, e.g., the Kabat numbering scheme, the Chothia numbering scheme, or a combination of Kabat and Chothia (see, e.g. Sequences of Proteins of Immunological Interest, U.S. Department of Health and Human Services (1991), eds. Kabat et al.; Lazikani et al., (1997) J. Mol. Bio. 273:927-948); Kabat et al., (1991) Sequences of Proteins of Immunological Interest, 5th edit., NIH Publication no. 91-3242 U.S. Department of Health and Human Services; Chothia et al., (1987) J. Mol. Biol. 196:901 -917; Chothia et al., (1989) Nature 342:877-883; and Al-Lazikani et al., (1997) J. Mol. Biol. 273:927-948; Annals of the New York Academy of Sciences, 764, 47-49 (1995); Nucleic Acids Research, 25, 206-211 (1997).

A "**human antibody**" or "**human antibody fragment**", as used herein, is an antibody and antibody fragment having variable regions in which both the framework and CDR regions are from sequences of human origin. Human antibodies can also be isolated from synthetic libraries or from transgenic mice (e.g. Xenomouse, OmniMouse, Harbour Mouse, ATX-Gx Mouse, Trianni Mouse) provided the respective system yield in antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also

is derived from such sequences. Human origin includes, e.g., human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik et al., (2000) J Mol Biol 296:57-86).

A "**humanized antibody**" or "**humanized antibody fragment**" is defined herein as an antibody molecule, which has constant antibody regions derived from sequences of human origin and the variable antibody regions or parts thereof or only the CDRs are derived from another species. For example, a humanized antibody can be CDR-grafted, wherein the CDRs of the variable domain are from a non-human origin, while one or more frameworks of the variable domain are of human origin and the constant domain (if any) is of human origin.

The term "**chimeric antibody**" or "**chimeric antibody fragment**" is defined herein as an antibody molecule, which has constant antibody regions derived from, or corresponding to, sequences found in one species and variable antibody regions derived from another species. Preferably, the constant antibody regions are derived from, or corresponding to, sequences found in humans, and the variable antibody regions (e.g. VH, VL, CDR or FR regions) are derived from sequences found in a non-human animal, e.g. a mouse, rat, rabbit or hamster.

The term "**antigen binding arm**", as used herein, refers to a component part of an antibody fragment of the invention that has an ability to bind specifically a target molecule of interest. The antigen binding arm is a complex of variable domain sequences (VL and VH), including the CDRs and the framework regions of an immunoglobulin light and heavy chain, and constant domain sequences (CL and CH) of an immunoglobulin light and heavy chain.

The "**hinge region**", "**hinge sequence**", and variations thereof, as used herein, includes the meaning known in the art, which is illustrated in, for example, Janeway et al. , Immuno Biology: the immune system in health and disease, (Elsevier Science Ltd. , NY) (4th ed. , 1999).

The phrase "**truncated hinge region**", as used herein, refers to a polypeptide comprising parts, but not all, of a hinge sequence. The truncated hinge region is capable of linkage to the "first" Fc polypeptide. If the wild type hinge sequence is not present, the remaining sequence in the "second" Fc polypeptide would comprise a component that is capable of linkage to the "first" Fc polypeptide. For example, said component can be a modified residue or an added cysteine residue capable of forming a disulfide linkage .

A "**knob**" refers to at least one amino acid side chain which projects from the interface of a first Fc polypeptide and is therefore positionable in a compensatory hole in the adjacent interface (i.e. the interface of a second Fc polypeptide) so as to stabilize the heteromultimer, and thereby favor heteromultimer formation over homomultimer formation, for example. The knob may exist in the original interface or may be introduced

synthetically (e.g. by altering nucleic acid encoding the interface). Normally, a nucleic acid encoding the interface of the first polypeptide is altered to encode the knob. To achieve this, the nucleic acid encoding at least one "original" amino acid residue in the interface of the first polypeptide is replaced with nucleic acid encoding at least one "import" amino acid residue which has a larger side chain volume than the original amino acid residue. It will be appreciated that there can be more than one original and corresponding import residue. The upper limit for the number of original residues which are replaced is the total number of residues in the interface of the first polypeptide.

A "**hole**" refers to at least one amino acid side chain which is recessed from the interface of a second Fc polypeptide and therefore accommodates a corresponding knob on the adjacent interface of a first Fc polypeptide. The hole may exist in the original interface or may be introduced synthetically (e.g. by altering nucleic acid encoding the interface). Normally, nucleic acid encoding the interface of the second polypeptide is altered to encode the hole. To achieve this, the nucleic acid encoding at least one "original" amino acid residue in the interface of the second polypeptide is replaced with nucleic acid encoding at least one "import" amino acid residue which has a smaller side chain volume than the original amino acid residue. It will be appreciated that there can be more than one original and corresponding import residue. The upper limit for the number of original residues which are replaced is the total number of residues in the interface of the second polypeptide.

The knob is "**positionable**" into the hole which means that the spatial location of the knob and hole on the interface of a first Fc polypeptide and second Fc polypeptide respectively and the sizes of the knob and hole are such that the knob can be located into the hole without significantly perturbing the normal association of the first and second polypeptides at the interface. Since knobs do not typically extend perpendicularly from the axis of the interface and have preferred conformations, the alignment of a knob with a corresponding hole relies on modeling the knob/hole pair based upon a three-dimensional structure such as that obtained by X-ray crystallography or nuclear magnetic resonance (NMR) . This can be achieved using widely accepted techniques in the art.

The term “**isolated antibody**” or “**isolated antibody fragment**” refers to an antibody or antibody fragment that is substantially free of other antibodies or antibody fragments having different antigenic specificities. Moreover, an isolated antibody or antibody fragment may be substantially free of other cellular material and/or chemicals. Thus, in some aspects, antibodies provided are isolated antibodies, which have been separated from antibodies with a different specificity. An isolated antibody may be a monoclonal antibody. An isolated antibody may be a recombinant monoclonal antibody. An isolated antibody that specifically binds to an epitope, isoform or variant of a target may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., species homologs).

The term "**recombinant antibody**" or "**recombinant antibody fragment**", as used herein, includes all antibodies or antibody fragment that are prepared, expressed, created or segregated by means not existing in nature. For example, antibodies isolated from a host cell transformed to express the antibody, antibodies selected and isolated from a recombinant, combinatorial human antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of all or a portion of a human immunoglobulin gene, sequences to other DNA sequences or antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom. Preferably, such recombinant antibodies have variable regions in which the framework and CDR regions are derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies can be subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. A recombinant antibody may be a monoclonal antibody.

The term "**monoclonal**" as used herein has the meaning typically ascribed to it in the art, namely an antibody or an antibody fragment (or its corresponding functional fragment) arising from a single clone of an antibody-producing cell, recognizing a single epitope on the antigen bound.

As used herein, an antibody "**binds specifically to**", "**specifically binds to**", is "**specific to/for**" or "**specifically recognizes**" an antigen, such as human Met, if such antibody is able to discriminate between such antigen and one or more reference antigen(s), since binding specificity is not an absolute, but a relative property. For example, a standard ELISA assay or standard flow cytometry assay can be carried out. The scoring may be carried out by standard color development (e.g. secondary antibody with horseradish peroxide and tetramethyl benzidine with hydrogen peroxide) or by binding of a secondary antibody labeled with PE or another dye or marker. The reaction in certain wells is scored by the optical density (OD), for example, at 450 nm or by mean or median fluorescence intensity (MFI) in flow cytometry. Typical background (=negative reaction) may be 0.1 OD; typical positive reaction may be 1 OD. Background and positive reaction MFI are highly dependent on instrument settings. The difference positive/negative can be more than 10-fold. Typically, determination of binding specificity is performed by using not a single reference antigen, but a set of about three to five unrelated antigens, such as milk powder, BSA, transferrin or the like. For flow cytometry various antigen-negative cells can be used. An antibody that specifically binds to an antigen may however have cross-reactivity to the respective orthologous antigen from other species (e.g., species homologs). In certain embodiments such cross-reactivity to an orthologous antigen is even preferred.

As used herein, an antibody has "**cross-reactivity**" or is "**cross-reactive**" if it binds to the orthologous antigen from other species. For example, an antibody is cross-reactive if it binds to human Met and to cynomolgus Met.

As used herein, the term "**affinity**" refers to the strength of interaction between the polypeptide and its target at a single site. Within each site, the binding region of the polypeptide interacts through weak non-covalent forces with its target at numerous sites; the more interactions, the stronger the affinity.

The term "**epitope**" includes any proteinaceous region which is specifically recognized by an antibody or antibody fragment thereof or otherwise interacts with a molecule. Generally, epitopes are of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally may have specific three-dimensional structural characteristics, as well as specific charge characteristics. As will be appreciated by one of skill in the art, practically anything to which an antibody can specifically bind could be an epitope.

The term "**domain**" or "**protein domain**" refers to a region of a protein's polypeptide chain that forms a functional unit and/or independently forms a three-dimensional structure.

"**Compositions**" or of the present disclosure may be used for therapeutic or prophylactic applications. The present disclosure, therefore, includes a pharmaceutical composition containing an antibody or antibody fragment as disclosed herein and a pharmaceutically acceptable carrier or excipient therefore. In a related aspect, the present disclosure provides a method for treating inflammatory diseases, autoimmune diseases, hematologic malignancies and potentially other diseases. Such method contains the steps of administering to a subject in need thereof an effective amount of the pharmaceutical composition that contains an antibody or antibody fragment as described herein.

The present disclosure provides therapeutic methods comprising the administration of a therapeutically effective amount of an antibody or antibody fragment as disclosed herein to a subject in need of such treatment. A "**therapeutically effective amount**" or "**effective amount**", as used herein, refers to the amount of an anti-Met antibody necessary to elicit the desired biological response. In accordance with the subject disclosure, the therapeutic effective amount is the amount of an anti-Met antibody necessary to treat and/or prevent a disease.

"**Administered**" or "**administration**" includes but is not limited to delivery of a drug by an injectable form, such as, for example, an intravenous, intramuscular, intradermal or subcutaneous route or mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestible solution, capsule or tablet. Preferably, the administration is by an injectable form.

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

"**Preventing**" or "**prevention**" refers to a reduction in risk of acquiring or developing a disease (i.e., causing at least one of the clinical symptoms of the disease not to develop in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset). "Prevention" also refers to methods which aim to prevent the onset of a disease or its symptoms or which delay the onset of a disease or its symptoms.

"**Subject**" or "**species**" or as used in this context refers to any mammal, including rodents, such as mouse or rat, and primates, such as cynomolgus monkey (Macaca fascicularis), Marmoset monkey (Callithrix jacchus), rhesus monkey (Macaca mulatta) or humans (Homo sapiens). Preferably, the subject is a primate, most preferably a human.

The term "**effector function**" refers to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Non-limiting examples of antibody effector functions include C1 q binding and complement dependent cytotoxicity (CDC); Fc receptor binding and antibody-dependent cell-mediated cytotoxicity (ADCC) and/or antibody- dependent cellular phagocytosis (ADCP); down regulation of cell surface receptors (e.g. B cell receptor); and direct cell activation or direct cell inhibition.

"**Antibody-dependent cell-mediated cytotoxicity**" or "**ADCC**" refers to a form of cytotoxicity in which antibodies bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g. NK cells, neutrophils, and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes/macrophages express FcγRI, FcγRII, and FcγRIII.

"**Complement-dependent cytotoxicity**" or "**CDC**" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) of the present disclosure, which are bound to their cognate antigen.

"**Antibody-dependent cellular phagocytosis**" or "**ADCP**" refers to a mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells.

Throughout this specification, unless the context requires otherwise, the words "**comprise**", "**have**" and "**include**" and their respective variations such as "**comprises**", "**comprising**", "**has**", "**having**", "**includes**" and "**including**" will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The terms "**engineered**" or "**modified**" as used herein includes manipulation of nucleic acids or polypeptides by synthetic means (e.g., by recombinant techniques, in vitro peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques). Preferably, the antibodies or antibody fragments according to the present disclosure are engineered or modified to improve one or more properties, such as antigen binding, stability, half-life, effector function, immunogenicity, safety and the like.

"**Variant**" as used herein refers to a polypeptide that differs from a reference polypeptide by one or more modifications for example amino acid substitutions, insertions or deletions. Variant polypeptides typically retain most of the properties of the reference polypeptide, e.g. binding to the target antigen, but introduce a novel, additional feature or property, e.g. the variant polypeptide has a higher affinity to the target antigen compared to the reference polypeptide or the variant polypeptide is a humanized version of the reference polypeptide.

The term "**amino acid mutation**" as used herein is meant to encompass amino acid substitutions, deletions, insertions, and modifications. Any combination of substitution, deletion, insertion, and modification can be made as long as the final construct possesses the desired characteristics, e.g., reduced binding to an Fc receptor. Amino acid sequence deletions and insertions include N-and/or C-terminal deletions and insertions of amino acid residues. Particular amino acid mutations are amino acid substitutions. Amino acid substitutions include replacement by non-naturally occurring amino acids or by naturally occurring amino acid derivatives of the twenty standard amino acids. Amino acid mutations can be generated using genetic or chemical methods well known in the art. Genetic methods may include site-directed mutagenesis, PCR, gene synthesis and the like. It is contemplated that methods of altering the side chain group of an amino acid residue by methods other than genetic engineering, such as chemical modification, may also be useful. Various designations may be used herein to indicate the same amino acid mutation. For example, a substitution of glycine at position 237 of the antibody Fc region to alanine can be indicated as 237A, G237A, or Gly237Ala.

The term "**EC50**" as used herein, refers to the concentration of an antibody or antibody fragment, which induces a response in an assay half-way between the baseline and maximum. It therefore represents the antibody or ligand concentration at which 50% of the maximal effect is observed.

The term "**Ka**", as used herein refers to the association rate of a particular antibody-antigen interaction

The term "**Kd**" as used herein, refers to the dissociation rate of a particular antibody-antigen interaction. Kd values for antibodies can be determined using methods well established in the art.

The term "**KD**" as used herein, refer to the dissociation constant of a particular antibody-antigen interaction, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration. A preferred method for determining the Kd of an antibody is by using surface plasmon resonance, preferably using a biosensor system, such as a Biacore system, or by using biolayer interferometry with the Octet BLI instrument.

The terms "**inhibition**" or "**inhibit**" or "**reduction**" or "**reduce**" or "**neutralization**" or "**neutralize**" refer to a decrease or cessation of any phenotypic characteristic (such as binding or a biological activity or function) or to the decrease or cessation in the incidence, degree, or likelihood of that characteristic. "Inhibition", "reduction" or "neutralization" needs not to be complete as long as it is detectable using an appropriate assay. In some embodiments, by "reduce" or "inhibit" or "neutralize" is meant the ability to cause a decrease of 20% or greater. In another embodiment, by "reduce" or "inhibit" or "neutralize" is meant the ability to cause a decrease of 50% or greater. In yet another embodiment, by "reduce" or "inhibit" or "neutralize" is meant the ability to cause an overall decrease of 75%, 85%, 90%, 95%, or greater.

The term "**antagonistic**" antibody as used herein refers to an antibody or antibody fragment that interacts with an antigen and partially or fully inhibits or neutralizes a biological activity or function or any other phenotypic characteristic of a target antigen.

A "**wild-type**" protein is a version or variant of the protein as it is found in nature. An amino acid sequence of a wildtype protein, e.g., a Fc region of a human IgG1 antibody, is the amino acid sequence of the protein as it occurs in nature. Due to allotypic differences, there can be more than one amino acid sequence for a wildtype protein. For example, there are several allotypes of naturally occurring human IGg1 heavy chain constant regions (see, e.g., Jeffries et al. (2009) mAbs 1 :1).

The "**Fc region**" is used to define the C-terminal region of an immunoglobulin heavy chain. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain. Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human IgG heavy chain Fc region

is usually defined to extend from Cys226, or from Pro230, to the C-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991. Various Fc modifications are commonly used. For a review see for example Antibodies (2020) 9: 64.

The terms "**silent**", "**silenced**" as used herein in the context of a silenced antibody or an antibody comprising a silencing mutation refers to a mutation in the Fc domain of such antibody which decreases, partially or wholly, binding to one or more cell surface Fcgamma receptors, thereby reducing or dampening, and in some embodiments abrogating substantially completely, one or more Fc-mediated antibody effector functions, such as ADCC, ADCP, and CDC complement response (see, e.g., Kang and Jung, Experimental and Molecular Medicine (2019) 51:138). Silenced effector functions can be obtained by mutation in the Fc region of the antibody and have been described in the Art (e.g.,, Strohl, Biotechnology 20: 685-91 for LALA and N297A; Baudino et al., J. Immunol. 181: 6664-69 for D265A). Other exemplary Fc silencing mutations include amino acid substitutions at one of more of positions E233, L234, L235, G236, N297, P331 and P329 (see e.g. U.S. Pat. Nos.6,737,056, 7,332,581; WO 2004/056312, WO2021/234402, and Shields, R. L. et al., J. Biol. Chem.276 (2001) 6591-6604). Silencing mutations also include (numbering according EU index) the LALA (L234A/L235A), the PA-LALA (L234A/L235A/P329A) and the PG-LALA (L234A/L235A/P329G) mutations, as well as the AEASS mutations (L234A/L235E/G237A/A330S/P331S).

Embodiments of the invention

In the following description, numerous specific details are given to provide a thorough understanding of the embodiments. The embodiments can be practiced without one or more of the specific details, or with other methods, components, materials, etc. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the embodiments.

The instant disclosure concerns a novel therapeutic agent for the treatment of a tumor and/or metastasis.

It is estimated that more than 200,000 patients per year are "MET-addicted", and Met inhibition may potentially result in remission of the disease. Considering that mutations accumulate with aging

and over the next 20 years the ageing population will increase, the burden of cancer is expected to rise and to have a high impact on global healthcare resources for patients' management. Genetic alterations responsible for "MET-addiction" have been found in gastric, oesophageal, colorectal, renal, and lung carcinoma, melanoma, and brain tumors. Moreover, selection of MET genetic lesions has been found as an acquired mechanism of resistance to a number of other targeted therapies in colorectal and non-small cell lung cancer (NSCLC).

The role of Met in metastasis is also associated with the ability of Met in helping cells to adapt to a harsh environment. The metastatic capabilities driven by Met not only depend on genetic and epigenetic alterations, but also on paracrine secretion of HGF by tumor stromal tissue which is composed by a large variety of cell types, including fibroblasts, resident epithelial cells, pericytes, myofibroblasts, vascular and lympho-vascular endothelial cells, and infiltrating cells of the immune system.

Met is nowadays recognized as a cancer-specific target for: (i) personalized treatment of tumors with MET mutations/amplifications ('addicted to MET'); (ii) for prevention/reversion of Met-driven primary and secondary resistance to other targeted cancer therapies; and (iii) for prevention/reversion of Met-driven invasive/metastatic phenotype.

Monovalent antibodies are for example described in WO2005/063816 and Proc Natl Acad Sci USA (2013) 110, E2987. A "one-arm" anti-Met antibody named "hOA-DN30" is described in WO2020/074459. hOA-DN30 is a highly stable humanized, monovalent antibody which blocks Met activation by a shedding mechanism, including (i) removal of Met from the cell surface by 'shedding' of the ectodomain; (ii) sequestration of the HGF ligand; (iii) inhibition of the homo- or hetero-dimerization of Met receptor at the membrane; and (iv) stimulation of receptor degradation. The present disclosure provides significant additional improvements to hOA-DN30. The introduction of a silencing mutations not only resulted in an antibody with effects associated with respective mutations, but also provides an additional important safety aspect - the abrogation of Fc gamma receptor reduces the residual risk of MET dimerization through immune cell Fc gamma receptor mediated super-crosslinking on the tumor cell surface.

Anti-Met antibody fragments

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein humanized VL domain is fused to the human CL domain in the N- to C-terminal direction.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human

CH3 constant domain,

wherein humanized VL domain is fused to the human CL domain in the N- to C-terminal direction, and

wherein the humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the human hinge region is fused to the CH2 domain that is fused to the human CH3 domain in the N- to C-terminal direction, wherein the human hinge region is truncated at the N-terminus.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide

comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein humanized VL domain is fused to the human CL domain in the N- to C-terminal direction,

wherein the humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction, and

wherein the human hinge region is fused to the CH2 domain that is fused to the human CH3 domain in the N- to C-terminal direction, wherein the human hinge region is truncated at the N-terminus.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the humanized VL domain has an amino acid sequence as set forth in SEQ ID No.: 13.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the humanized VH domain has an amino acid sequence as set forth in SEQ ID No.: 14.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the humanized VL domain has an amino acid sequence as set forth in SEQ ID No.: 13, and

wherein the humanized VH domain has an amino acid sequence as set forth in SEQ ID No.: 14.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the human CL domain is a human light kappa type domain.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second

human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the human hinge region and the human constant domains CH1, CH2 and CH3 are from a human IgG1.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the human CL domain is a human light kappa type domain, and

wherein the human hinge region and the human constant domains CH1, CH2 and CH3 are from a human IgG1.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide

comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the two Fc polypeptides are linked through intermolecular disulfide bonds at the hinge region.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the first Fc polypeptide and the second Fc polypeptide meet at an interface, and one between the first and the second Fc polypeptide comprises a knob at the interface, and the other between the first and the second Fc polypeptide comprises a hole at the interface, wherein the knob is positionable into the hole.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human

light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein either the first or the second Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries an amino acid mutation at position 389, wherein the original amino acid at position 389 has been mutated to import an amino acid having a larger side chain volume than the original amino acid; and wherein the other Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries three amino acid mutations at positions 389, 391 and 438, wherein the original amino acids have been mutated to import amino acids having smaller side chains volume than the original amino acids, wherein the amino acid numbering is according to the EU numbering scheme of Kabat.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second

human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the original amino acids at positions 389, 391 and 438 are threonine, leucine and tyrosine respectively; and wherein in the first or the second Fc polypeptide the threonine in position 389 has been mutated to tryptophan; and wherein in the other Fc polypeptide the threonine at position 389 has been mutated to serine, the leucine at position 391 has been mutated to alanine and the tyrosine at position 438 has been mutated to valine.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the human CL domain has an amino acid sequence as set forth in SEQ ID No.: 15 and the human CH1 domain has an amino acid sequence as set forth in SEQ ID No.: 16.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the first human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 17, and the second human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 18.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the anti-Met antibody fragment when bound to Met induces shedding of an extracellular domain of Met.

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the Fc region of the first Fc polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A (according to EU index), or the mutations L234A, L235E, G237A, A330S and P331S (according to EU index).

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the Fc region of the first FC polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A (according to EU index).

In certain embodiments, the present disclosure relates to an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the first polypeptide comprises the amino acid sequence of SEQ ID No. 19, the second polypeptide comprises the amino acid sequence of SEQ ID No. 20, and the third polypeptide comprises the amino acid sequence of SEQ ID No. 18.

Nucleic acids

In certain embodiments, the present disclosure relates to an isolated nucleic acid encoding an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide

comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain. In certain embodiments, said humanized VL domain is fused to the human CL domain in the N- to C-terminal direction. In certain embodiments, said humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction. In certain embodiments, said human hinge region is fused to the CH2 domain that is fused to the human CH3 domain in the N- to C-terminal direction, wherein the human hinge region is truncated at the N-terminus. In certain embodiments, said humanized VL domain is fused to the human CL domain in the N- to C-terminal direction, said humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction, and said human hinge region is fused to the CH2 domain that is fused to the human CH3 domain in the N- to C-terminal direction, wherein the human hinge region is truncated at the N-terminus. In certain embodiments, said humanized VL domain has an amino acid sequence as set forth in SEQ ID No.: 13. In certain embodiments, said humanized VH domain has an amino acid sequence as set forth in SEQ ID No.: 14. In certain embodiments, said humanized VL domain has an amino acid sequence as set forth in SEQ ID No.: 13, and said humanized VH domain has an amino acid sequence as set forth in SEQ ID No.: 14. In certain embodiments, said human CL domain is a human light kappa type domain. In certain embodiments, said human hinge region and the human constant domains CH1, CH2 and CH3 are from a human IgG1. In certain embodiments, said human CL domain is a human light kappa type domain, and said human hinge region and the human constant domains CH1, CH2 and CH3 are from a human IgG1. In certain embodiments, the two Fc polypeptides are linked through intermolecular disulfide bonds at the hinge region. In certain embodiments, the first Fc polypeptide and the second Fc polypeptide meet at an interface, and one between the first and the second Fc polypeptide comprises a knob at the interface, and the other between the first and the second Fc polypeptide comprises a hole at the interface, wherein the knob is positionable into the hole. In certain embodiments, either the first or the second Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries an amino acid mutation at position 389, wherein the original amino acid at position 389 has been mutated to import an amino

acid having a larger side chain volume than the original amino acid; and wherein the other Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries three amino acid mutations at positions 389, 391 and 438, wherein the original amino acids have been mutated to import amino acids having smaller side chains volume than the original amino acids, wherein the amino acid numbering is according to the EU numbering scheme of Kabat. In certain embodiments, the original amino acids at positions 389, 391 and 438 are threonine, leucine and tyrosine respectively; and in the first or the second Fc polypeptide the threonine in position 389 has been mutated to tryptophan; and wherein in the other Fc polypeptide the threonine at position 389 has been mutated to serine, the leucine at position 391 has been mutated to alanine and the tyrosine at position 438 has been mutated to valine. In certain embodiments, said human CL domain has an amino acid sequence as set forth in SEQ ID No.: 15 and said human CH1 domain has an amino acid sequence as set forth in SEQ ID No.: 16. In certain embodiments, the first human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 17, and the second human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 18. In certain embodiments, said anti-Met antibody fragment when bound to Met induces shedding of an extracellular domain of Met. In certain embodiments, the Fc region of the first Fc polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A (according to EU index), or the mutations L234A, L235E, G237A, A330S and P331S (according to EU index). In certain embodiments, the Fc region of the first FC polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A (according to EU index).

In certain embodiments, the present disclosure relates to an isolated nucleic acid encoding an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the first polypeptide comprises the amino acid sequence of SEQ ID No. 19, the second polypeptide comprises the amino acid sequence of SEQ ID No. 20, and the third polypeptide comprises the amino acid sequence of SEQ ID No. 18.

In certain embodiments, the present disclosure relates to an isolated nucleic acid encoding an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) and wherein the first polypeptide has the amino acid sequence of SEQ ID No. 19;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) and wherein the second polypeptide has the amino acid sequence of SEQ ID No. 20, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain, and wherein the third polypeptide has the amino acid sequence of SEQ ID No. 18.

In certain embodiments, the present disclosure relates to an isolated nucleic acid encoding an anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) and wherein the first polypeptide is encoded by the nucleic acid sequence

of SEQ ID No. 21;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) and wherein the second polypeptide is encoded by the nucleic acid sequence of SEQ ID No. 22, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain, and wherein the third polypeptide is encoded by the nucleic acid sequence of SEQ ID No. 23.

In certain embodiments, the present disclosure relates to a composition comprising two or more recombinant nucleic acids which collectively encode the anti-Met antibody fragment disclosed herein.

Medical uses and manufacturing

Therapeutic compositions comprising the active ingredient of the instant disclosure, i.e. the humanized anti-Met antibody fragment a, can be prepared with physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed., 1980), in the form of aqueous solutions, lyophilized, or other dried formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers; antioxidants; preservatives; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids; monosaccharides, disaccharides, and other carbohydrates; chelating agents; sugars; salt-forming counter-ions; metal complexes and/or non-ionic surfactants. The formulations may also contain other active compound (s) as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the therapeutic activity of hOA-DN30 alone or in combination with the extracellular portion of human Met. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredient may also be entrapped in microcapsules prepared by means of techniques disclosed i.a. in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the active ingredients of the

The active ingredient of the present disclosure (and adjunct therapeutic agents) are administered by any suitable means, including parenteral, subcutaneous, intraperitoneal, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. The active ingredient of the instant invention can be suitably administered by pulse infusion, particularly with declining doses of the active ingredients. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic.

The active ingredient will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The active ingredient need not be, but may optionally be, formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of active ingredient (present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99 percent of the heretofore employed dosages.

For the treatment of disease, the appropriate dosage of the active ingredient will depend on the type of disease to be treated, the severity and course of the disease, whether the active ingredients are administered for preventive or therapeutic purposes, the patient's clinical history and response to the active ingredients of the invention are duly taken into consideration, and at the discretion of the attending physician.

The anti-Met antibody fragments of the present disclosure are suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 mg/kg to 30 mg/kg of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dose might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the

treatment is sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody fragment would be in the range from about 0.05 mg/kg to about 20 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

In certain embodiments, the present disclosure relates to the anti-Met antibody fragment disclosed herein for use in the treatment of a tumor and/or metastasis.

In certain embodiments, the present disclosure relates to the anti-Met antibody fragment disclosed herein for use in the treatment of a tumor and/or metastasis in a patient carrying genetic alterations of the MET gene.

In certain embodiments, the present disclosure relates to the anti-Met antibody fragment disclosed herein for use in the treatment of a tumor and/or metastasis in a patient carrying a wild-type MET gene.

In certain embodiments, the present disclosure relates to a process for the manufacturing of an anti-Met antibody fragment disclosed herein, the process comprising the following steps: (i) synthesis of cDNA sequences of the first, second and third polypeptides constituting the anti-Met antibody fragment, (ii) insertion of the three cDNA sequences into one or more plasmids, wherein the plasmid(s) is(are) suitable for expression in a mammalian cell line, (iii) transient or stable co-transfection of a mammalian cell line with the plasmid(s), (iv) collection of the culture supernatant, (v) purification by affinity chromatography of the anti-Met antibody fragment.

Examples

Example 1: Rational and selection of possible silencing mutation

While Met is a validated target for the treatment of tumors and metastasis, there is still the possibility that respective targeting agent trigger the undesired dimerization and activation of the c-Met receptor, thereby initiating the downstream immune cell mediated signalling cascade. It was therefore rationalized that the abrogation of Fc gamma receptor binding could potentially prevent such unwanted downstream action. A prerequisite for such an approach is the a targeting agent that is not dependent on Fc effector function. In the present disclosure one such exemplary targeting agent was chosen to test this hypothesis. VERT001 is an anti-Met antibody fragment with an Fc effector-independent mode of action. It was hypothesized that a silent version of VERT001 may be a suitable candidate. Engineering of antibodies and antibody fragment however always bears the risk that not only a new function is introduced into the respective molecule, but that the molecule also looses other functions, such as efficacy, stability or other properties, that could limit the usefulness of the resulting derivative for therapeutic development.

Generated were derivatives of VERT001 in which certain silencing mutations were engineered into the Fc region of VERT001. VERT001 with the PA-LALA mutation yielded in VERT002. VERT001 with the AEASS mutation yielded in VERT004. VERT001, VERT002 and VERT004 are compared in the following examples.

Amino acid sequences of the binders are shown in the following Table.

Table 1:

CDR	**Nomenclature**	**SEQ ID No.**	**Amino acid sequence**
LCDR1	IMGT	2	QSVDYDGGSY
	Kabat	3	KASQSVDYDGGSYMS
LCD2	IMGT	4	AAS
	Kabat	5	AASNLES
LCDR3	IMGT	6	QQSYEDPLT
	Kabat	6	QQSYEDPLT
HCDR1	IMGT	7	GYTFTSYW
	Kabat	8	SYWIH
HCDR2	IMGT	9	INPSSGRT

	Kabat	10	EINPSSGRTNYNEKFKN
HCD3	IMGT	11	ASRGY
	Kabat	12	RGY
VL		13	DIVLTQSPDSLAVSLGQRATINCKASQSVD YDGGSYMSWFQQKPGQPPKLLIYAASNLES GVPARFSGSGSGTDFTLTISSLQAEDVATY YCQQSYEDPLTFGGGTKVEIK
VH		14	QVQLQQSGAEVKKPGASVKLSCKASGYTFT SYWIHWVRQAPGQGLEWIGEINPSSGRTNY NEKFKNRVTVTVDKSTSTAYMELSSLTSED SAVYYCASRGYWGQGTTLTVSS
CL		15	RTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
CH1		16	ASTKGPSVFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPS NTKVDKKV
First Fc		17	EPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALAAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
Second Fc; VERT-002, third polypeptide		18	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALAAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLSCAVK

		GFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLVSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
VERT-002, first polypeptide	19	DIVLTQSPDSLAVSLGQRATINCKASQSVD YDGGSYMSWFQQKPGQPPKLLIYAASNLES GVPARFSGSGSGTDFTLTISSLQAEDVATY YCQQSYEDPLTFGGGTKVEIKRTVAAPSVF IFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC
VERT-002, second polypeptide	20	QVQLQQSGAEVKKPGASVKLSCKASGYTFT SYWIHWVRQAPGQGLEWIGEINPSSGRTNY NEKFKNRVTVTVDKSTSTAYMELSSLTSED SAVYYCASRGYWGQGTTLTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALAAPIEKT ISKAKGQPREPQVYTLPPSRDELTKNQVSL WCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
VERT-002, first nucleic acid	21	GATATCGTGCTAACCCAATCACCCGATTCTCTGGCTGTCT CTCTGGGCCAGCGGGCCACCATCAATTGCAAGGCTAGCCA ATCCGTGGACTACGACGGTGGCAGCTACATGTCCTGGTTC CAACAAAAGCCCGGACAACCACCAAAGCTGCTGATCTATG CGGCTAGCAACCTGGAGTCGGGGGTTCCAGCAAGGTTCAG CGGCAGTGGAAGTGGAACAGACTTCACCTTGACAATCTCC AGCCTCCAGGCTGAGGACGTGGCCACCTATTATTGCCAAC AAAGCTATGAGGATCCTCTGACATTCGGCGGCGGTACTAA GGTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTC

		ATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTG CCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGA GGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCG GGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGG ACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAA AGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTC ACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGT
VERT-002, second nucleic acid	22	CAGGTTCAACTCCAACAAAGTGGTGCCGAGGTCAAGAAAC CTGGAGCCAGCGTCAAACTGTCGTGCAAAGCTAGCGGCTA TACCTTCACTTCCTACTGGATCCACTGGGTCAGACAGGCC CCAGGGCAGGGCTTGGAGTGGATTGGAGAAATTAACCCAT CTAGTGGAAGAACCAACTACAACGAAAAGTTTAAGAATCG CGTGACCGTGACCGTGGATAAGTCTACTAGCACCGCATAC ATGGAGCTCAGCTCACTCACATCTGAGGACAGCGCTGTCT ATTATTGCGCTTCCCGCGGCTACTGGGGCCAGGGCACTAC CTTAACTGTTTCCTCCGCTAGCACCAAGGGCCCATCGGTC TTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCA CAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGA ACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC GGCGTGCACACCTTCCCGGCCGTCCTACAGTCCTCAGGAC TCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAG CTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG CCCAGCAACACCAAGGTGGACAAGAAGGTTGAGCCCAAAT CTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACC TGAAGCCGCTGGGGGACCGTCAGTCTTCCTCTTCCCCCCA AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGG TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGA GGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA CGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGA CTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC AAAGCCCTCGCCGCCCCCATCGAGAAAACCATCTCCAAAG CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCC CCCATCCCGGGACGAGCTGACCAAGAACCAGGTCAGCCTG TGGTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCG TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAA GACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC

		CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGC AGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG GGCAAA
VERT-002, third nucleic acid	23	GACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAAG CCGCCGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACC CAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACA TGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCA AGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGC CAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGC TGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC CCTCGCCGCCCCCATCGAGAAAACCATCTCCAAAGCCAAA GGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCAT CCCGGGACGAGCTGACCAAGAACCAGGTCAGCCTGTCCTG CGCCGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG TGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCA CGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCGT GAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACA ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGCAA A

VERT-002 has the following mutations in the CH2 domain: L234A, L235A and P329A. VERT-004 has the following mutations in the CH2 domain: L234A, L235E, G237A, A330S and P331S.

Example 2: General methods employed in the present study

Example 2.1 Purity evaluation by size exclusion chromatography (SEC)

Monomeric content was assessed by HP-SEC analysis using an Agilent 1260 infinity II. Liquid chromatography separation was performed through an advance bio SEC column (300A 2.7µm, 4.6 * 300mm). Chromatography was performed at room temperature (RT) with a flow rate of 0.35 ml/min. As the mobile phase 200mM sodium phosphate buffer pH 7.0 was used. Prior to chromatography

samples were diluted to 1mg/ml in 200mM sodium phosphate buffer pH7.0. Samples were centrifuged for 5 min at 75000 rcf (except for the 55°C and pH stressed samples which were filtered). 80 µl of supernatant were transferred to a HPLC vial with insert. The chromatograms were manually integrated with ChemStation software. A MW standard was used as column quality control.

Example 2.2 Purity evaluation by strong cation exchange chromatography (SCX)

The size variant profile was analyzed by SCX chromatography using an Agilent 1260 infinity II. Liquid chromatography separation was performed through an Agilent bio SCX NP1.7 SS column (4.6 * 50mm). Chromatography was performed at room temperature (RT) with a flow rate of 0.8 ml/min. As the mobile phase A (loading) 20 mM sodium phosphate buffer pH 6.0 was used, as mobile phase B (elution) 20 mM sodium phosphate buffer pH 6.0 + 0.5M NaCl. The gradient applied was as follows:

Table 2:

Gradient	Time (min)	%A	%B
	2	100	0
	14	80	20
	15	80	20
	16	100	0
	18	100	0

For the sample preparation, the solution formulations were diluted to 20 mM sodium phosphate buffer pH 6.0. Samples were centrifuged for 5 min at 75000rcf (except for the 55°C and pH stressed samples which were filtered). 80 µl of supernatant were transferred to a HPLC vial with insert. The chromatograms were manually integrated with ChemStation software.

Example 2.3 Purity evaluation by hydrophobic interaction chromatography (HIC)

Purity of the samples assessed by hydrophobic interaction chromatography using an Agilent 1260 infinity II. The liquid chromatography separation was performed through an Agilent bio HIC 4.6*100mm, 3.5um. Chromatography was performed at room temperature (RT) with a flow rate of 0.8

ml/min. As the mobile phase A (loading) 50mM sodium phosphate buffer pH 7.0 + 2M $(NH_4)_2SO_4$ was used, as mobile phase B (elution) 50mM sodium phosphate buffer pH 7.0. The gradient applied was as follows:

Table 3:

Gradient	Time (min)	%A	%B
	Starting condition	60	40
	30	0	100
	35	0	100
	40	60	40
	50	60	40

For the sample preparation, the solution formulations were diluted to to 1mg/mL in 50mM Sodium phosphate buffer pH7.0. Samples were centrifuged for 5 min at 75000rcf (except for the 55°C and pH stressed samples which were filtered). 80 µl of supernatant were transferred to a HPLC vial with insert. The chromatograms were manually integrated with ChemStation software.

Example 2.4 Purity evaluation by reverse phase UPLC-MS

Purity of the samples assessed by UPLC-MS analysis using a Waters Acquity H class + system connected to a PDA and a single quadrupole detector from waters SQD2. The liquid chromatography separation was performed through a BEH column C4 300A, 2.1x50mm.

For the sample preparation, the solution formulations were diluted to 0.5mg/ml in water (100 µl final volume). Mass lynx was used for data acquisition and data processing. Max Ent1 was used to determine the molecular mass of the protein. LC-MS parameters are shown in the following Table.

Table 4:

LC method for intact mass	
Analytical column	BEH C4 300A 1.7um, 2.1 x 50mm

Mobile phase A	100 Water + 0.1 % FA		
Mobile phase B	100% ACN + 0.1% FA		
Column temperature	80°C		
Flow rate	0.5mL		
UV detection	Scan 192 -498 nm		
Injection volume	10 µL		
Inlet file	Inlet file desalt 10min		
Gradient	Times	%A	%B
	0	95	5
	1.5	95	5
	7	40	60
	8	95	5
	10	95	5
Column temperature	80°C		
Flow rate	0.5mL		
UV detection	Scan 192 -498 nm		
Injection volume	10 µL		
MS method			
MS file	MS SCAN pos 10min 1000_3000 scan05		
MS tune file	MS 2kV 80V 500C 1000L_hr new calib		
Cone voltage	80V		
Capillary voltage	2kV		
Source temperature	150 °C		

Example 2.5 Measurement of thermal unfolding via Differential Scanning Fluorimetry

Samples were characterized by Differential Scanning Fluorimetry (DSF) using Sypro Orange Dye (SO). In brief, protein samples were mixed with the SO Dye in PBS buffer, then a temperature gradient was applied, and fluorescence was monitored as a function of temperature (°C). Under heat proteins start to unfold and expose hydrophobic core residues which the SO interacts with, and an increase of fluorescence can be measured. Sypro Orange Dye 5000X was diluted to a concentration of 10x in PBS. All samples were diluted to a concentration of 2mg/mL in PBS buffer then mixed 1:1 in volume with

SO 10x to obtain a final concentration of sample 1mg/mL and SO 5X in PBS. Each sample was measured in triplicate.
Samples are transferred in a 96-wells PCR plate and RT-PCR thermocycler C1000 (Biorad) was used to apply a temperature gradient from 25°C to 95°C with 0.5°C increase steps each 10 seconds. The first derivate of the curve was used to determine the melting temperature (Tm) which gives information about the conformational stability of the protein samples. SO fluorescence at 25°C (starting point of the temperature ramp) relative to each sample was used to give qualitative information about the initial folding state.

Example 2.6 ELISA binding assay

The microplates were first coated with. To do so, recombinant human cMET ECD His (SinoBiological PN: 10692-H08H) was diluted to a final concentration of 1 µg/ml in PBS in F96 IMMUNOPLATE MAXISORP plates to a final volume of 50 µl and incubate at 4°C overnight. Then plates were blocked to minimize non-specific binding. Coating solution was removed, then 100 µl of blocking buffer (MSD blocking buffer) or 5% BSA in PBS was added on each well and incubated for 1h at room temperature on an orbital shaker at 600rpm. Blocking buffer was then washed 3x with MSD wash buffer or PBS-0.05% P20.

Next, 50 µl of diluted primary antibodies (VERT001, VERT002 and VERT-04; titrated in another plate using ASSAY BUFFER = PBS 1x, 0.1% BSA w/v, 0.02% P20) were added to each well. Concentrations used were 400, 100, 25, 12.5, 6.25, 0.39, 0.098, 0.024, 0.0061, 0.0015, 0.00038 ug/ml. All samples were prepared in duplicate. The plates were incubated for 1 hour at room temperature on an orbital shaker at 600 rpm followed by 3x washing with MSD wash buffer or PBS-0.05% P20. Diluted secondary antibody (Goat a-human IgG Fc Secondary Antibody, HRP 1:20000) was then added to each well (50 µl) and incubated for 1hour at room temperature on an orbital shaker at 600 rpm followed by 3x washing with MSD wash buffer or PBS-0.05% P20. Finally, for the detection TMB (3,3',5,5'-tetramethylbenzidine) was added (50ul/well) and incubated for 1 to 5 min (until blue color appears) followed by addition of equal volume of stopping solution (2 M H_2SO_4). The optical density was then measured at 450 nm in a plate reader. Absorbance at 450 nm was plotted against concentration of primary antibody and data were fitted to a 4PL equation to obtain the EC50.

Example 2.7 Proliferation assay

Hs746T cells were purchased from ATCC (cat. Number HTB135), sub-cultured in Dulbecco's MEM (4.5 g/L glucose + GlutaMAX, Gibco 31966) supplemented with 1% Sodium pyruvate (Gibco, 11360) and 10% heat inactivated FBS (PAN Biotech P30-1909), incubated at 37°C, 5% CO_2. EBC-1 cells were purchased from JCRB (cat. Number JCRB0820), sub-cultured in RPMI (+ glutamine, + 25mM HEPES, Gibco 22400) with 10% heat inactivated FBS, incubated at 37°C, 5% CO_2.

Proliferation assays were performed on white 96-well assay plates (ThermoFisher 136101) with cell plating (5,000 cells per well for Hs746T cells and 2,000 cells per well for EBC-1 cells) on day 0, compound addition on day 1, and survival on day 4. On day 1, compounds were diluted to a concentration of 2000 µg/ml and 9-point dose responses (1:4 dilution series) were prepared in culture medium before adding to the cells (final starting concentration of dose response, 200 µg/ml). All samples were tested in duplicates. Viability was assessed using CellTiter-Glo 2.0 Luminescent Cell Viability assay kit (Promega) that measures cellular ATP content. CellTiter-Glo was added (dilution 1:10) and incubated with the cells for 20 min in the dark on a plate shaker at 600rpm. Luminescence was recorded with an EnVision multimode plate reader (Perkin Elmer). On day 1, viability of untreated cells was measured to quantify background and subtract this value to day 4 viability results. IC50 was determined by nonlinear regression using a four-parameter (variable slope) curve fit, implemented in GraphPad Prism (version 9.3.0) to fit dose-response data (after subtraction of day 1 background level).

Example 2.8 Stability studies

For stability studies, 6 x 1ml tubes of each solution containing the respective antibody were taken out of -80°C freezer and thawed at room temp protected from light. Each solution was then aliquoted into 3R glass vial (400 µl per vial for VERT001 and VERT002 and 350 µL for VERT004 due to a lower starting volume) under laminar flow hood to keep the solutions sterile. The vials were then capped with the rubber stopper and closed with a flip-off seals.

3 vials per antibody were stored at 5°C, 3 vials at 40°C and 1 vial at -80°C. 1 vial was used as T0 measurement. The time points were as follows: D0, Week1 (W1), W2 and W4. At each time point a new aliquot of the respective antibody vials was taken out of -80°C freezer to use as assay standard in

all the characterization techniques. This allows to evaluate the deviation and variability inter runs between each time points.

3ml of each solution was kept for a forced degradation study (pH and heat). The samples were kept for 2 days at 5°C before the start of the study.

Example 2.9 Forced degradation studies

Example 2.9.1 pH stability

Sample were prepared as described in Example 2.8. Then 850 µL of each solution was pH adjusted to pH3 with 1M HCl or to pH9 with 1M NaOH. Vials were kept at RT, protected from light for 5 days. At D0 and D2, samples were taken and stored at -80°C. At D5, the samples were directly stored at -80°C. On the day of analysis, the aliquots were sterile filtered to rend them compatible for cell assay. Therefore, no centrifugation step was applied for the various LC sample prep.

Example 2.9.2 Temperature stability

Sample were prepared as described in Example 2.8. Then 300 µl of each solution was aliquoted into Eppendorf tubes and placed into an Eppendorf thermoblock set at 55°C for 5 days without shaking and protected from light. On the day of analysis, the samples were sterile filtered to rend them compatible for cell assay. Therefore no centrifugation step was applied for the various LC sample prep.

Example 2.9.3 Freeze-thaw stability

Sample were prepared as described in Example 2.8. Aliquots for each antibody were taken out from -80°C and left at RT, protected from light for 60-90 minutes. Once fully thawed, the aliquot was stored at -80°C again. A total of 3 F/T (freeze/thaw) cycles was performed.

Example 2.10 Concentration measurements

Protein concentrations were measured by A280 using a nanophotomer NP80 (Implen). The extinction coefficient (l/g*cm) was 1.48 for VERT001 and VERT004 and 1.49 for VERT002. 2uL of sample was pipetted onto the sample window. PBS buffer was used to perform the blank. Concentrations reported are the average of 2 measurements.

Example 3: Biophysical properties and developability assessment

Example 3.1 Comparison of the formats

VERT-001 (wild-type Fc), VERT-002 (PA-LALA) and VERT-004 (AEASS) were compared in various assays without exposure to any stress conditions.

Example 3.1.1 SEC analysis

In SEC analysis all formats showed a high monomeric content of more than 99%. The percentage of the high molecular weight (HMW) and the low molecular weight (LMW) species was essentially identical for all 3 samples testes. See Table 5.

Table 5:

	MONOMER	HMW	LMW
VERT-001	99.3	0.7	-
VERT-008	99.2	0.8	-
VERT-004	99.3	0.7	-

Example 3.1.2 SCX analysis

SCX chromatograms are shown in Figure 1. In SCX analysis all three formats showed a similar charged species distribution. VERT-004 appears to be slightly more acidic, probably due to the L to E mutation. Table 6 shows the percentage of main species and the acidic and basic species as determined by SCX analysis.

Table 6:

	%Main species	%Acidic	%Basic
VERT-001	56	25	19
VERT-002	63	25	12
VERT-004	61	24	15

Example 3.1.3 HIC analysis

The HIC chromatograms of all three formats look highly identical. All three formats show a relatively early retention time suggesting low hydrophobicity.

Example 3.1.4 Reversed phase UPLC- MS analysis

On the RP chromatogram, VERT-004 shows a somewhat different profile compared to VERT-001 and VERT-002 indicated by the presence of multi-peaks. See Figure 2.

The masses of the main UV peak were determined by MS for each antibody and are summarized in in the following Table.

Table 7:

	Intact Mass (Da)	
	Main peak	Secondary peak

VERT-001	100120	100280 (40% of main peak)
VERT-002	99900	100060 (40% of main peak)
VERT-004	100105	100265 (100% of main peak)

The mass differences between the various formats are in good agreement with their mutations:

ΔVERT-001- VERT-002 = 220Da, which corresponds to the mutation PLL (SEQ ID No. 24) to AAA (SEQ ID No. 25): 2x 110Da.

ΔVERT-001- VERT-004 = 15Da, which corresponds to the mutation LLGAP (SEQ ID No. 26) to AEASS (SEQ ID No. 27): 2x 6Da.

A detailed mass analysis of each individual peak present in VERT-001, VERT-002 and VERT-004 was performed. A summary and tentative species assignment for VERT-004 is depicted in Figure 3. VERT-001 and VERT-002 also contains a small level of truncated HC, and a small portion of unidentified species at 90 and 89kDa respectively corresponding to the MW – 10kda for both antibodies.

Example 3.1.5 Differential Scanning Fluorimetry

Thermal unfolding curves for the antibodies VERT-001, VERT-002 and VERT-004 were recorded via Differential Scanning Fluorimetry and melting temperatures (Tm) and observed fluorescence at 25°C (RFU t0) were determined. Tm for VERT-001 and VERT-002 are 68°C and 68.5°C. respectively. while VERT-004 shows a lower Tm of 62.5°C. All Fc formats starts at around 4000 RFU in initial fluorescence. Results are summarized in the following Table.

Table 8:

	Tm (°C)	RFU t0
VERT001	68	4141
VERT002	68.5	4409
VERT004	62.5	4545

Example 3.1.6 Binding to human c-Met ECD

Binding of VERT001, VERT002 and VERT004 to the ECD of c-Met was measured in an ELISA assay. Binding ELISA curves are shown in Figure 4. EC50'S determined are shown in the following Table.

Table 9:

Binder	EC50 [ng/ml]
VERT001	80
VERT002	66
VERT004	96

Binding curves of all three binders, VERT001, VERT002 and VERT004, were similar. VERT002 showed the highest affinity.

Example 3.1.7 Affinity determination by BLI

The binding affinity of VERT-001, VERT-002 and VERT-004 was compared by affinity determination via BLI. Results are shown in Table 10.

Table 10:

	Affinity, K_D [nM]
VERT-001	5.68
VERT-002	2.82
VERT-004	3.43

Of all three antibodies, VERT-002 showed the highest affinity

Example 3.1.8 Proliferation

The anti-proliferative activity of VERT-001, VERT-002 and VERT-004 was measured in a respective assay, utilizing Hs746T cells and EBC-1 cells. Results are shown in Figure 5. IC50'S determined are shown in the following Table.

Table 11:

IC50 values (nM)	Hs746T	EBC-1
VERT-001 WT	60.0	78.2
VERT-002 PALALA	64.5	67.6
VERT-004 AEASS	57.9	70.7
VERT-001 WT (repeat)	87.6	90.9
VERT-002 PALALA (repeat)	100.8	82.4
VERT-004 AEASS (repeat)	87.4	63.0

Example 3.2 Stability studies

Samples were prepared as described in Example 2.8.

Example 3.2.1 Visual inspection of the stability samples

The stability samples at 5°C and 37°C for all 3 antibody formats remained visually clear and particles free throughout the 4 weeks. The concentration remained unchanged over the 4 weeks at 5 and 37 °C.

Example 3.2.2 Aggregate and fragment analysis via SEC

The formation of monomer, HMW and LMW content was determined over the incubation period via SEC analysis. Results are shown in Figure 6.

A very slight monomer decrease associated with a HMW increase is observed for VERT002 (0.4%) and VERT004 (0.6 %) at 5°C. At 37°C, VERT-001 and VERT-002 have a similar slow to moderate kinetic of monomer decrease accompanied by aggregation (HMW increase) and fragmentation (LMW increase). A higher aggregation kinetic is observed for VERT004 as indicated by a rapid decrease of

monomer content and increase in HMW species. The VERT-004 LMW increase follows a similar slope as for VERT-001 and VERT-002.

Example 3.2.3 Charge variant analysis via SCX

The formation of charge variants over the incubation period was analyzed via strong cation exchange chromatography. Results are shown in Figure 7. At 5°C, the charge variant profile remained stable for all formats. When exposed to 37°C, all formats showed the same trend in terms of charge variant evolution with an increase of acidic species over time, likely corresponding to deamidation and a reduction in main peak.

Example 3.2.4 Stability analysis via RP-HPLC

The chemical stability was monitored over the incubation period by RP with UV and MS detection. No major degradation was observed for any of the formats. A small additional peak (RT = 4.48 min) was however detected for all the antibodies after 4 weeks at 37°C, possibly attributable to fragmentation.

Example 3.2.5 Stability analysis via DSF

Stability of all three antibodies after incubation at 5°C and 37°C for 4 weeks was compared to reference material freshly thawed was studied by DSF. The melting temperature for all samples is identical to the one observed at time point zero (T0) regardless of the storage condition. All FC formats show an initial fluorescence around 4000 RFU. Results are summarized in the following Table.

Table 12:

	Tm(°C)	RFU t0
Ref Mat	68	3687
VERT001 5C 4W	68	3815
VERT001 37C 4W	68	3957
VERT002 5C 4W	68.5	3726
VERT002 37C 4W	68.5	4024
VERT004 5C 4W	62.5	3913
VERT004 37C 4W	62.5	4252

Example 3.2.6 Binding to human c-Met ECD

Binding of VERT001, VERT002 and VERT004 to the ECD of c-Met after incubation at 5°C and 37°C for 4 weeks was compared to reference material freshly thawed was measured in an ELISA assay. When samples were incubated at 37°C for 4 weeks, there was a trend for minor decrease in binding potency for VERT-001, VERT-002 and VERT-004 when compared to the assay standard. The same samples incubated for the same time at 5°C seem to be stable and no decrease in binding potency can be observed when compared to the assay standard. A comparison of the binding of the samples relative to the reference material is shown in Figure 8.

Example 3.2.7 Proliferation

The anti-proliferative activity of VERT001, VERT002 and VERT004 after incubation at 5°C and 37°C for 4 weeks was compared to freshly thawed reference material in Hs746T cells and EBC-1 cells. Overall, the anti-proliferative activity of all samples is in the expected range throughout the study, but a slight shift in potency is observed at week 4 in both cell lines (higher IC50 in Hs746T and lower IC50 in EBC-1). IC50'S determined are shown in the following Table.

Table 13:

IC50 values (nM)	D0		Week 1	Week 2	Week 4
Hs746T	60.0	87.6	63.9	93.5	87.9
EBC-1	78.2	90.9	77.5	92.4	33.5

Example 3.3 Forced degradation studies

Example 3.3.1 Visual inspection of pH stressed samples

The pH stressed samples were all clear and free of particles over 5 days. The concentration dropped slightly for VERT002 and VERT004 at pH3 at D0 and D5, likely due to the dilution upon pH adjustment.

Example 3.3.2 Analysis of chemical degradation of pH stressed samples by RP

pH stress at pH3 and pH9 did not lead to any significant degradation of the antibodies, as indicated by the presence of a main peak corresponding to the correct intact mass, and also a constant peak height between D0 and D5. A low level of degradation can be observed after 5 days at pH 3 for VERT-002 and VERT-004 as suggested by the presence of small peaks eluting before the intact antibody. The deconvoluted intact mass (in Da) for the pH stressed samples is shown in the following Table.

Table 14:

	VERT-001	VERT-002	VERT-004
T0	100120	99900	100105
pH3 D5	100125	99910	100115
pH9 D5	100125	99900	100110

Example 3.3.3 Analysis of samples stressed at pH3 by SCX and SEC

At pH3, all formats strongly aggregate already at D0. No peak was detected by SCX analysis of the samples. As RP confirmed that the protein is still present, the absence of detection by SCX is likely due to the strong aggregation preventing peak elution. An exemplary chromatogram (VERT002) is shown in Figure 9.

Example 3.3.4 Analysis of samples stressed at pH9 by SCX and SEC

At pH9 a slight increase of HMW is observed for all three antibodies. Also observed was a small increase in acidic species. No differences could be observed between CERT001, VERT002 and VERT004.

Example 3.3.5 Analysis of stressed samples by DSF

The impact of pH on the VERT samples stability was analyzed by DSF. VERT-002 and VERT-004 incubated at pH9 do not show visible changes in initial fluorescence or Tm. When both samples were incubated at pH3, very high initial fluorescent at t0 can be detected and Tm cannot be determined. This is indicative of lower stability (aggregation and or misfolding) of VERT-002 and VERT-004 at acidic pH while a basic pH seems not to impact those samples in DSF. The observation at pH3 correlates well with the SEC results showing aggregation. Results are summarized in the following Table.

Table 15:

	Tm(°C)	RFU t0
Ref Mat	68	3849
VERT001 pH3	-	-
VERT001 pH9	-	-
VERT002 pH3	ND	15245
VERT002 pH9	68	3989
VERT004 pH3	ND	15962
VERT004 pH9	63	4268

As for temperature stress, incubation at 55°C for 5 days showed much higher initial fluorescence (RFU at t0) compared to the assay standard for all samples, probably attributable to aggregation or misfolding events. None of the antibodies were impacted by 3x freezing/throwing (F/T) cycles as it can be observed by the unaffected Tm and RFU at t0. Results are summarized in the following Table.

Table 16:

	Tm(°C)	RFU t0
Ref Mat	68	3849
VERT001 55°C	68	11475
VERT001 3xF/T	68	3756
VERT002 55°C	68.5	11876
VERT002 3xF/T	68.5	3836
VERT004 55°C	63	11422
VERT004 3x F/T	63	3785

Example 3.3.6 Analysis of samples stressed at 55°C

All antibodies were subjected to 55°C for 5 days. VERT001 and VERT002 had some filaments formed at the end of the incubation period, while VERT004 had particles. After filtration, only VERT004 showed a marked reduction of the antibody concentration (4.4mg/ml). Under these stress condition, all formats show an increase in aggregate (higher HMW) and in fragmentation (higher LMW). See the following Table.

Table 17:

		Monomer	HMW	LMW
VERT001	**D0**	99.3	0.7	-
	D5 55°C	92.6	5.1	2.3
VERT002	**D0**	99.2	0.8	-
	D5 55°C	91.1	7.6	1.4
VERT004	**D0**	99.3	0.7	-
	D5 55°C	94.2	4.2	1.6

In RP chromatography analysis, some slight impurities/fragments could be detected which elute just before 4.50 min for VERT-002 and VERT-004 D5 55°C. The mass deconvolution suggests a fragment at ~14 kDa. VERT-001 could not be analyzed due to limited sample volume remaining. Interestingly, a decrease in the initial fragments/impurities (truncated FC, LC, mispaired fragments) could be observed for VERT-004 (and too a smaller extent also for VERT-002).The 55°C condition leads to similar observation than incubation for 4 weeks at 37°C.

Example 3.3.7 Analysis of samples subjected to freeze/thaw cycles

VERT001, VERT002 and VERT004 were subjected to 3 freeze (-80°) / thaw (RT) cycles. No change were observed in IEX, SEC or RP. The following Table summarizes the percentage of monomer, HMW and LMW as determined by SEC analysis.

Table 18:

		Monomer	HMW	LMW
VERT001	**D0**	99.3	0.7	-
	FT	99.4	0.6	-
VERT002	**D0**	99.2	0.8	-
	FT	99.2	0.8	-
VERT004	**D0**	99.3	0.7	-
	FT	99.2	0.8	-

Example 3.3.8 Analysis of the anti-proliferative activity of the stressed samples

All three antibodies, VERT001, VERT002 and VERT004, stressed at different conditions were tested for their anti-proliferative activity.

At pH3, all antibodies completely lost their anti-proliferative activity on Hs746T and EBC-1 cells while the activity of stressed antibodies at pH9 remained similar to the activity of the reference material.

The anti-proliferative activity of the binders stressed at 55°C for 5 days was tested on Hs746T only. The activity of VERT-001 and VERT-002 stressed at 55°C remained in the expected range, whereas VERT-004 lost its anti-proliferative activity.

Results are shown in Figure 10. IC50's determined for cell line Hs746T are shown in Table 18, IC50's determined for cell line EBC-1 in Table 19.

Table 19:

Hs746T

IC50 values (nM)		pH3	pH9	55°C
Assay Standard	93.7			
VERT-001 WT		-	109.0	142.9
VERT-002 PALALA		-	76.1	196.4
VERT-004 AEASS		-	110.1	-

Table 20:

EBC-1

IC50 values (nM)		pH3	pH9
Assay Standard	98.2		
VERT-001 WT		-	90.6
VERT-002 PALALA		-	76.9
VERT-004 AEASS		-	83.46

The antiproliferative activity of VERT-001, VERT-002 and VERT-004 after 3 freeze/thaw (F/T) cycles did not show any deviation from the activity of the assay standard after F/T cycles.

Example 4: Pharmacological and efficacy studies

Example 4.1: Efficacy and pharmacology in a Hs746T xenograft model

The in vivo anti-tumor potency of VERT-001 was compared to VERT-002 and VERT-004 in a Hs746T model (MET exon 14 skipping and MET amplification) sub-cutaneously (s.c.) implanted into female hairless SCID mice. All variants were tested at the same dose level, 20 mg/kg given twice per week (BIW) intravenously (i.v.). Results are shown in Figure 11. All variants tested showed a full and comparable potency at 20 mg/kg.

Dose-response for the anti-tumor efficacy of VERT-002 in vivo was evaluated further in the Hs746T CDX model. VERT-002 was administered i.v. at doses of 10 and 20 mg/kg twice per week and 20 mg/kg once per week, whilst an isotype control was administered at 20 mg/kg twice per week, for up to 4 weeks (Figure 12). VERT-002 shows dose-dependent tumor growth inhibition (TGI), with full tumor regression when administered at the top dose of 20 mg/kg twice per week. By Day 35, 6 of 9 mice from the highest dose group were tumor-free, as reflected by the very low mean tumor volume levels at Day 35.

The 20 mg/kg twice per week treated mice were monitored for over 30 days after dosing had ended, where the majority of the treatment group (6/9 mice) remained tumor-free. The 3/9 mice with residual tumors at the end of dosing period (Day 32) were the only mice with tumor regrowth during post-dose monitoring.

Example 4.2: Efficacy and pharmacology in a EBC-1 xenograft model

VERT-002 was further profiled in vivo with EBC-1 cells (harboring MET amplification) s.c. implanted into female Balb/c nude mice. VERT-002 was administered i.v. at doses of 5, 10, and 20 mg/kg twice per week, and 20 mg/kg once per week, whilst an isotype control was administered at 20 mg/kg twice per week (Figure 13, panel A and B). VERT-002 showed clear dose-dependent TGI, with full tumor regression seen at 20 mg/kg twice per week, which is consistent with the results from the Hs746T CDX model. In addition, VERT-002 induced significant increases in levels of soluble MET ectodomain (sMET ECD), which is dose-dependent, reaching a plateau at around day 6 of treatment (Figure 13, panel C). Interestingly, in response to VERT-002 dosing, sMET ECD levels mirror the tumor regression profile, indicating less ECD is shed as the tumor is shrinking (tumor regression occurring from around the same time point, day 7). Similar results for VERT-002 dose-response on TGI and sMET ECD was seen also in a chronic study (single dose) in the EBC-1 model (data not shown).

Claims

1. An anti-Met antibody fragment comprising a single antigen binding arm and a silenced Fc region, wherein the Fc region comprises a complex of a first and a second Fc polypeptide, wherein the antibody fragment comprises:

(i) a first polypeptide comprising a humanized light chain variable (VL) domain, and one human light chain constant (CL) domain, wherein the humanized VL domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 3, 5 and 6;

(ii) a second polypeptide comprising a humanized heavy chain variable (VH) domain, a human heavy chain constant CH1 domain and the first Fc polypeptide, wherein the first Fc polypeptide comprises a hinge region, a human constant CH2 domain and a human CH3 constant domain, wherein the humanized VH domain contains three complementary determining regions (CDRs) having amino acid sequences as set forth in SEQ ID No.: 8, 10 and 12, and

(iii) a third polypeptide comprising the second human Fc polypeptide, wherein the second human Fc polypeptide comprises a human hinge region, a human constant CH2 domain and a human CH3 constant domain,

wherein the Fc region of the first FC polypeptide and the second Fc polypeptide comprise the mutations L234A, L235A and P329A (according to EU index).

2. The anti-Met antibody fragment according to claim 1, wherein humanized VL domain is fused to the human CL domain in the N- to C-terminal direction.

3. The anti-Met antibody fragment according to claim 1 or 2, wherein the humanized VH domain is fused to the human CH1 domain, that is fused to the human hinge region, that is fused to the human CH2 domain, that is fused to the human CH3 domain in the N- to C-terminal direction.

4. The anti-Met antibody fragment according to any one of claims 1-3, wherein the human hinge region is fused to the CH2 domain that is fused to the human CH3 domain in the N- to C-terminal direction, wherein the human hinge region is truncated at the N-terminus.

5. The anti-Met antibody fragment according to any one of claims 1-4, wherein the humanized VL domain has an amino acid sequence as set forth in SEQ ID No.: 13.

6. The anti-Met antibody fragment according to any one of claims 1-5, wherein the humanized VH domain has an amino acid sequence as set forth in SEQ ID No.: 14.

7. The anti-Met antibody fragment according to any one of claims 1-6, wherein the first Fc polypeptide and the second Fc polypeptide meet at an interface, and one between the first and the second Fc polypeptide comprises a knob at the interface, and the other between the first and the second Fc polypeptide comprises a hole at the interface, wherein the knob is positionable into the hole, wherein either the first or the second Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries an amino acid mutation at position 389, wherein the original amino acid at position 389 has been mutated to import an amino acid having a larger side chain volume than the original amino acid; and wherein the other Fc polypeptide comprises a mutated CH3 constant domain, wherein the mutated CH3 constant domain carries three amino acid mutations at positions 389, 391 and 438, wherein the original amino acids have been mutated to import amino acids having smaller side chains volume than the original amino acids, wherein the amino acid numbering is according to the EU numbering scheme of Kabat.

8. The anti-Met antibody fragment according to claim 7, wherein the original amino acids at positions 389, 391 and 438 are threonine, leucine and tyrosine respectively; and wherein in the first or the second Fc polypeptide the threonine in position 389 has been mutated to tryptophan; and wherein in the other Fc polypeptide the threonine at position 389 has been mutated to serine, the leucine at position 391 has been mutated to alanine and the tyrosine at position 438 has been mutated to valine.

9. The anti-Met antibody fragment according to any one of claims 1 to 8, wherein the human CL domain has an amino acid sequence as set forth in SEQ ID No.: 15 and the human CH1 domain has an amino acid sequence as set forth in SEQ ID No.: 16.

10. The anti-Met antibody fragment according to any one of claims 1 to 9, wherein the first human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 17, and the second human Fc polypeptide has an amino acid sequence as set forth in SEQ ID No.: 18.

11. The anti-Met antibody fragment according to any one of claims 1 to 10, wherein the first polypeptide comprises the amino acid sequence of SEQ ID No. 19, the second polypeptide comprises the amino acid sequence of SEQ ID No. 20, and the third polypeptide comprises the amino acid sequence of SEQ ID No. 18.

12. Isolated nucleic acid encoding the anti-Met antibody fragment of any of claims 1 to 11.

13. A composition comprising two or more recombinant nucleic acids which collectively encode the anti-Met antibody fragment of any of claims 1 to 11.

14. An anti-Met antibody fragment according to any one of claims 1 to 11 for use in the treatment of a tumor and/or metastasis.

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