SUPPLEMENTARY METHODS

Antibody germlining and engineering

The VH and Vλ regions of WT52 (1) were germlined by genetic engineering of their framework regions to the closest human germline sequence found in the ImMunoGeneTics (IMTG) Repertoire (Z14238-IGHV4-30-4*01 for VH and L27697-IGLV2-18*02 for Vλ; see the University of Zürich online sequence collection at www.bioc.uzh.ch/plueckthun/antibody/Sequences/Germlines/IMGT_hVH.html and _hVL.html). Deviating amino acids were mutated by PCR using overlapping, degenerated oligonucleotide primers encoding both the human and llama residues as described (2), creating synthetic phage libraries expressing germlined Fab fragments. Germlined functional Fab fragments were selected as described (3). The selected germlined Fab was re-assembled with human IgG1 and lambda light chain constant domains as described (1) to generate the germlined G52 antibody. G52 was further engineered using NHance™ technology (4). Mutations encoding the H433K/N434F amino acid changes in the CH3 region were introduced by site-directed mutagenesis using a QuickChange® Site-Directed Mutagenesis Kit (Stratagene). PCR amplification was carried out using Phusion High-Fidelity DNA Polymerase (Thermo Scientific). To obtain an afucosylated antibody with enhanced ADCC activity, G52-HN was produced in the POTELLIGENT® GS CHOK1SV Chinese hamster cell line (Lonza) that lacks the *FUT8* gene encoding α-1,6-fucosyl transferase. The resulting afucosylated IgG1 antibody was named ARGX-111. G52 antibody variants for murine studies were generated as described (WT52-E, ref. 5; WT52-D., ref. 6). All antibodies were produced and purified as described (1). Concentration and purity of all purified mAbs was determined by measuring optical density at 280 nm and by SDS-PAGE. An irrelevant IgG1 with known concentration (Sigma Aldrich) was used as control in all experiments.

Receptor binding assays

Antibody binding to MET was performed by ELISA as described (1). Simian MET was purchased from Sino Biological. ARGX-111 and G52 binding to human FcRn (provided by Sally Ward, Texas University) was determined by Surface Plasmon Resonance (SPR) at pH 6.0 and pH 7.4 using a BIACORE 3000 apparatus (GE Healthcare) as described (4). ARGX-111, G52, WT52-E, WT52-D binding to human FcγRIIIa, human and mouse FcγRI and mouse FcγRIV (all from Sino Biological) were determined by ELISA. Maxisorb 96-well plates (Nunc) coated with neutravidin (100 ng/well; Thermo Scientific) were allowed to bind the appropriate biotinylated purified receptor for 1 hour at room temperature (25 ng/well) and then incubated with increasing mAb concentrations (0-200 nM). Antibody binding was revealed using an anti-human IgG Fab fragment conjugated with horseradish peroxidase (HRP; Sigma-Aldrich). Data were analyzed and fit using Prism software (GraphPad).

HGF-dependent and -independent in vitro assays

The ability of ARGX-111 to compete with HGF for binding to the MET extracellular domain and to inhibit HGF-dependent MET phosphorylation in A549 and NCI-H1437 cells was determined by ELISA as described (1). ARGX-111-mediated inhibition of HGF-independent MET phosphorylation in MKN-45 and EBC-1 cells was performed as described (1) with the following changes: no HGF was added; mAbs were incubated with cells for 24 hours instead of 15 minutes. ARGX-111-mediated inhibition of HGF-dependent cell migration was determined by a Boyden chamber assay. A549 cells were starved in DMEM containing 0.1% FBS for 24 hours and then seeded (8×10^4 cells) in the upper compartment of the Boyden chamber (Millipore) in presence or absence of 667 nM ARGX-111. After 1 hour, 0.65 nM recombinant human HGF (R&D Systems) was added in the lower compartment of the chamber, and cells were incubated for 24 hours at 37°C and 5% CO_2. At the end of the incubation period, filters were washed with PBS, fixed in 4% para-formaldehyde and stained in a VECTASHIELD® mounting medium

(Vector Laboratories). Cells were observed and counted (5 fields/filter) under an inverted microscope. The ability of ARGX-111 to inhibit HGF-dependent anchorage-independent growth was analyzed in a soft agar assay. NCI-H1437 cells were seeded in soft agar using 6-well plates (1×10^5 cells/well) in the presence or absence of 0.13 nM HGF (R&D Systems). Cells were incubated at 37°C in 5% CO_2 for three weeks and treated every third day with 67 nM ARGX-111. At the end of the experiment, cell colonies were stained with tetrazolium salts (Sigma-Aldrich), photographed, and quantified using Metamorph software (Molecular Devices). ARGX-111-mediated inhibition of HGF-induced branching morphogenesis in SV40 T-antigen-transformed LOC cells was performed as described (7).

Flow cytometry and MET down-regulation analysis

ARGX-111 binding to native MET was analyzed by flow cytometry as described (1). Analysis of ARGX-111-mediated MET down-regulation was performed by flow cytometry using MKN-45, EBC-1 and MDA-MB-231 cells. For time course experiments, cells were incubated with 330 nM ARGX-111 or an irrelevant human IgG1 (Sigma-Aldrich) for 0.3, 1, 3, 6 and 24 hours, and MET expression levels were determined by flow cytometry using allophycocyanin-conjugated anti-MET antibodies (R&D Systems; we have determined experimentally that this antibody does not compete with ARGX-111). For dose-response experiments, cells were incubated with increasing concentrations (0-1 µM) of ARGX-111 or an irrelevant IgG1, and MET expression was determined 24 hours later by flow cytometry as described above. For shedding and total MET protein analysis, MKN-45 cells were incubated with no factor, 330 nM irrelevant IgG1, 330 nM DN30 (8) or 330 nM ARGX-111 for 24 or 48 hours. MET shedding was analyzed by Western blotting of conditioned medium using an antibody directed against the extracellular portion of MET as described (9). Cell lysates were analyzed by Western blotting using anti-total MET antibodies (Invitrogen) and anti-actin antibodies (Santa Cruz Biotechnology). For MET kinase-dependency analysis, MKN-45, EBC-1 and MDA-MB-231 cells were incubated with 330 nM ARGX-111, 330 nM irrelevant IgG1,

500 nM JNJ-38877605 (Selleckchem), or 330 nM ARGX-111 plus 500 nM JNJ-38877605, and MET expression was determined 24 hours later by flow cytometry as described above. For HGF-induced MET internalization, MDA-MB-231 cells were incubated with 0 or 500 nM JNJ-38877605 as above. After 18 hours, cells were stimulated with no factor or 0.6 nM HGF, and then incubated for additional 6 hours prior to MET analysis by flow cytometry. In all cells, MET auto-phosphorylation and total MET levels were determined by Western blotting using anti-phospho MET antibodies (Cell Signaling) and anti-total MET antibodies (Invitrogen), respectively. For analysis of breast cancer stem cell markers, 6 week-old female NOD-SCID mice (Charles River) were injected orthotopically in the mammary fat pad with 2×10^6 MDA-MB-231 cells or 1×10^6 human primary mammary carcinoma cells (CRCM168, CRCM174, CRCM389; ref. 10) as described (1). When tumors reached a volume of 200-300 mm^3, mice were sacrificed and their tumors resected for processing. Single cell suspensions, obtained by enzymatic digestion of tumors as described (10), were co-stained as follows: (i) AldeFluor™ (Stemcell Technologies) and allophycocyanin-conjugated anti-MET antibodies (R&D Systems); (ii) phycoerithryn-conjugated anti-CD24 antibodies (R&D Systems), fluorescein isothiocyanate-conjugated anti-CD44 antibodies and allophycocyanin-conjugated anti-MET antibodies. Antibody binding was revealed by flow cytometry using a CyAn™ ADP analyzer (Beckman Coulter).

ADCP assays

For ADCP assessment, human monocytes were purified from heparinized whole blood from 3 different donors (Etablissement Français Du Sang) using a RosetteSep™ Human Monocyte Enrichment Cocktail (Stemcell Technologies). Cells were seeded (2×10^6 cells/ml) in 20 ml of X-VIVO15 medium with 500 U/ml GM-CSF (Miltenyi Biotec) and cultured for 15 days to generate monocyte-derived macrophages (MDMs). 786-O and MKN-45 target cells were labelled using a PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich) according to provided protocol. Stained cells (8×10^4) were pre-incubated for 1

hour with 5-fold serial dilutions of antibodies (ARGX-111, G52, irrelevant IgG1) starting at 133 nM, and then mixed with 2×10^4 MDM effector cells. After 1 hour, MDMs were labelled with fluorescein isothiocyanate-conjugated anti-CD11b antibodies (EXBIO) and analyzed by flow cytometry using a Guava EasyCyte™ Plus analyzer (Millipore). Phagocytosis was determined by quantifying double-stained cells. Experiments were performed in triplicate. Data were analyzed and fit using Prism software (GraphPad).

Study approval

Experiments involving mice were performed in the following facilities: U87-MG subcutaneous model, Aix-Marseille Université, IBDM, CNRS UMR 7288, Parc Scientifique de Luminy, Marseille, France; MKN-45 subcutaneous model, Department of Molecular Biomedical Research, Inflammation Research Center, Ghent University, Zwijnaarde, Belgium; orthotopic mammary carcinoma model, Laboratory of Experimental Therapy, Candiolo Cancer Institute - FPO, IRCCS, Candiolo, Italy. All animal studies were conducted in accordance with European directive 2010/63/EU and with national legislative regulations after local ethical approval. Protocols were approved by the following comimittees: Comité d'Éthique pour l'Expérimentation Animale de Marseille, Direction Départementale des Services Vétérinaires, Préfecture Des Bouches Du Rhône, Marseille, France; Animal Experimentation Ethical Committee, Faculty of Sciences, Ghent University, Ghent, Belgium; Animal Research Ethical Committee, Fondazione Piemontese per la Ricerca sul Cancro – ONLUS, Candiolo, Italy. Experiments involving human primary tumor samples were performed in the Laboratory of Experimental Therapy, Candiolo Cancer Institute - FPO, IRCCS, Candiolo, Italy. The protocol for human sample analysis was approved by the Comité de Protection des Personnes Sud-Méditerranée I, Marseille, France.

Statistical analysis

All quantitative data were analyzed and fit using Prism software (GraphPad) unless differently

stated. Bioluminescence signal in mice and isolated organs was analyzed using Living Image software (Perkin Elmer). Statistical significance was determined using a two-tailed homoscedastic Student's T-Test. A *p* value of less than 0.05 was considered statistically significant. In all figures, values are expressed as mean and error bars represent standard deviation or standard error of the mean as indicated.

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