The Selective Intravenous Inhibitor of the MET Tyrosine Kinase SAR125844 Inhibits Tumor Growth in \textit{MET}-Amplified Cancer

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Abstract

Activation of the MET/HGF pathway is common in human cancer and is thought to promote tumor initiation, metastasis, angiogenesis, and resistance to diverse therapies. We report here the pharmacologic characterization of the triazolopyridazine derivative SAR125844, a potent and highly selective inhibitor of the MET receptor tyrosine kinase (RTK), for intravenous administration. SAR125844 displayed nanomolar activity against the wild-type kinase (IC\textsubscript{50} value of 4.2 nmol/L) and the M1250T and Y1235D mutants. Broad biochemical profiling revealed that SAR125844 was highly selective for MET kinase. SAR125844 inhibits MET autophosphorylation in cell-based assays in the nanomolar range, and promotes low nanomolar proapoptotic and antiproliferative activities selectively in cell lines with MET gene amplification or pathway addiction. In two MET-amplified human gastric tumor xenograft models, SNU-5 and Hs 746T, intravenous treatment with SAR125844 leads to potent, dose- and time-dependent inhibition of the MET kinase and to significant impact on downstream PI3K/AKT and RAS/MAPK pathways. Long duration of MET kinase inhibition up to 7 days was achieved with a nanosuspension formulation of SAR125844. Daily or every-2-days intravenous treatment of SAR125844 promoted a dose-dependent tumor regression in \textit{MET}-amplified human gastric cancer models at tolerated doses without treatment-related body weight loss. Our data demonstrated that SAR125844 is a potent and selective MET kinase inhibitor with a favorable preclinical toxicity profile, supporting its clinical development in patients with \textit{MET}-amplified and MET pathway–addicted tumors. \textit{Mol Cancer Ther}; 14(2); 1–11. ©2014 AACR.

Introduction

The HGF/MET pathway plays an essential physiologic role during embryogenesis (1), and a more restricted role in tissue regeneration and damage repair in healthy adults (2, 3). In human cancer, the abnormal activation of the MET pathway is well documented. The MET tyrosine kinase subfamily, including MET and RON, is composed of dimer receptors with an extracellular \(\alpha\)-chain and a transmembrane \(\beta\)-chain. The intracellular domain of the \(\beta\)-chain is composed of a juxtamembrane region, involved in the regulation of the kinase activity and membrane recycling, a tyrosine kinase catalytic domain with Y1234 and Y1235 modulating this activity, and a C-terminal tail with Y1349 and Y1356 acting as docking sites for several intracellular effectors and adaptor proteins. Upon binding of the ligand, HGF, MET undergoes dimerization, autophosphorylation on Y1234, Y1235, Y1349, and Y1356, and promotes the activation of multiple downstream signaling pathways, including the RAS/MAPK, PI3K/AKT, and STAT3/5 pathways, ultimately leading to cell proliferation, survival, motility, invasion, and morphogenesis (4, 5).

MET was initially identified as part of the oncogenic fusion protein TPR-MET in cells treated by the N-methyl-N’-nitro-nitrosoguanidine carcinogen (6). Abnormal MET activation in patients with cancer is mediated by missense mutation and gene amplification, MET overexpression, and HGF-mediated paracrine or autocrine activation (reviewed in refs. 7 and 8). Mutations in the MET kinase domain, leading to an increased enzymatic activity, have been mainly described in patients with hereditary and sporadic papillary renal cell carcinoma (9). Alterations of \textit{MET} exon 14 (mutation or insertion/deletion) leading to in-frame loss of the juxtamembrane domain were reported in approximately 2% of patients with lung cancer (10). \textit{MET} gene amplification was reported in subsets of patients, including esophagogastric cancer (11–13), colorectal cancer (14), head and neck squamous cell cancer (15), glioblastoma (16) and EGFR-naïve non–small cell lung cancer (NSCLC; refs. 17, 18). MET gene amplification was

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also reported in 12% of patients with NSCLC with acquired resistance to EGFR kinase inhibitors (19–24) and in 40% (4 of 7 patients) of cetuximab or panitumumab-resistant patients with colorectal cancer (25). MET autocrine activation is thought to play a role in a subset of glioblastoma and acute myeloid leukemia (26, 27). Evidence of clinical benefit was reported with the MET antagonist onartuzumab/MetMAb antibody in a patient with chemotherapy-refractory, advanced metastatic gastric cancer with high MET expression and evidence of autocrine HGF stimulation (28). MET paracrine activation might be relevant in tumors with stromal cells expressing high level of HGF, and is also thought to promote the metastatic spread of cancer cells. More recently, HGF expression in the tumor stroma has been proposed as a resistance mechanism to BRAF inhibitors (29, 30).

Although the role of the MET/HGF pathway in human cancer is well documented, there is no yet approved MET selective inhibitor in MET-dependent cancer (31). Among advanced drugs, onartuzumab and rilotumumab, an anti-HGF antibody, are in phase III development in gastroesophageal cancer. Crizotinib, the MET, ALK, ROS, RON, and Aurora inhibitor approved for the treatment of patients with NSCLC positive for the EML4-ALK gene fusion, is in phase I development for MET-dependent cancers. Cabozantinib, the multikinase inhibitor of MET, VEGFR2, RET, KIT, and FLT3, approved for the treatment of RET-dependent medullary thyroid cancer, is in pivotal trials in metastatic renal cell cancer and advanced hepatocellular cancer. Other nonselective MET inhibitors (foretinib, golvatinib, MGGD-265) and antibodies targeting MET (LY-2875358, ABT-700) or HGF (flicautuzumab) are in phase I/II development. In addition, several selective MET kinase inhibitors are in phase I/II early clinical trials, including INCB28060, EMD1214063, and AMG-337. Here, we report the pharmacologic characterization of the triazolopyridazine derivative SAR125844, a novel, potent, reversible, and selective intravenously active MET kinase inhibitor in tumor models with MET gene amplification.

Materials and Methods

Compounds

SAR125844 was synthesized at Sanoﬁ, as described previously (refs. 32, 33; Fig. 1A), as well as the two reference MET kinase inhibitors JNJ-38877605 and PF-04217903. The compounds were formulated in 100% dimethyl sulfoxide as a 10 mmol/L stock solution for cell-based assays. For in vivo studies, SAR125844 was formulated in sulfobutylether-β-cyclodextrin 40% in acidified water for the solution formulation or PVPK17/DOC/Tris (0.4/0.08/0.016%) in dextrose adjusted at pH 7.4 for the nanosuspension formulation. SAR125844 solution and SAR125844 nanosuspension were given intravenously at 5 and 10 mL/kg, respectively. JNJ-38877605 was formulated in 98% PEG200, 2% PS80, and PF-04217903 was formulated in sulfobutylether-β-cyclodextrin 40% at pH 2.

Cell lines

All cell lines were obtained from ATCC, except MKN-45 and COLO-849 (Deutsche Sammlung von Mikroorganismen und Zellkulturen), SF-295 and HOP-92 (NCI, Bethesda, MD), OE33, ACHN, U-373 MG, and MDA-MB-231 (European Collection of Cell Cultures), EBC-1 and KP-4 (Japanese Collection of Research Bioresources Cell Bank), NHDF (Cambrex/Lonza), and HDMEC (Promocell), and maintained in culture conditions as recommended by the manufacturers. SNU-5 and Hs 746T cell lines were obtained from ATCC in 2006, and the dates of obtention of the other cell lines are indicated in Supplementary Table S1. The cell lines have not been subsequently authenticated since receipt.

Enzymatic homogeneous time-resolved fluorescence assay in MET wild-type and mutants

The MET kinase domains were produced at Sanoﬁ (except the MET Y1235D variant from Carna Biosciences, #08-198) and activated by autophosphorylation. SAR125844 was preincubated at room temperature with each enzyme for 30 minutes in a buffer containing 10 mmol/L MOPS-NaOH pH 7.0, 0.01% Tween 20, and 1 mmol/L dithiothreitol. The enzymatic reactions were initiated by the addition of a mix of 1 ng/μL of a biotinylated poly (glutamate-alanine-tyrosine) peptide (Cisbio, #61GATBLA), ATP and MgCl2. After 5-minute incubation at room temperature, the reactions were stopped by the addition of anti-phosphotyrosine monoclonal antibody (mAb) PI-66-Europium cryptate (Cisbio, #61166KLB) and streptavidin 61SAXLB (Cisbio). After two hours at room temperature, the emission signals at 620 and 665 nm were recorded with a GENios reader (TECAN), with an excitation wavelength of 320 nm. The percentage of inhibition versus non-treated sample was estimated using the emission ratios at 665/620 nm.

Cellular phospho-MET ELISA assay

MKN-45, Hs 746T, and SNU-5 cells were seeded in poly d-lysine 96-well plates in complete medium. Plates were incubated with increasing SAR125844 concentrations for 1 hour, cell lysates were generated using standard procedures and pMETY1230/1234/1235 level evaluated using a commercial ELISA (Life Technologies, KHO0281) as recommended by the supplier. IC50 values were calculated using the Biost@t-t-SPeed internal software and a 4-parameter logistic model.

Cellular assay on MET mutants

HEK-293T human renal cells were transiently transfected by vectors containing cDNA for the wild-type MET and the M1250T, Y1230H, Y1235D, L1195V, and D1228H MET mutants using Fugene reagent (Roche Applied Science). Forty-eight hours after transfection, cells were treated with SAR125844 for 1 hour, cell lysates generated, and pMETY1349/total MET levels evaluated with the multiprotot electrochemiluminescence MSD assay (MesoScale Discovery, N45126B-1). IC50 values corresponding to pMETY1349/total-MET inhibition were calculated using the Biost@t-t-SPeed internal software.

Cell proliferation assays

The antiproliferative activity of SAR125844 was evaluated in a panel of tumor cell lines in complete medium conditions, using two different read-outs, 13C thymidine incorporation, and intracellular ATP quantification. For thymidine incorporation, 10 μCi/mL 13C thymidine (NEN) was added to 96-well Cytostar microplates (Amersham) and the incorporation of radioactivity was measured using a Microbeta Wallac counter after a 96-hour treatment with SAR125844. For HGF-induced proliferation assays, after 24-hour serum deprivation 50 ng/mL HGF was added for 96 hours in the presence of 14C thymidine and SAR125844. For the ATP viability assay, cells were treated with SAR125844 for 96 hours, and viability was measured using CellTiter-Glo reagent.
SAR125844, a Selective Intravenous MET Kinase Inhibitor

Figure 1.
SAR125844 selectively inhibits proliferation in cells with MET gene amplification, promotes apoptosis and inhibits HGF-mediated tumor cell migration. A, chemical structure of SAR125844. B, dose-dependent inhibition of cell proliferation by SAR125844 exclusively in MET-driven tumor cell lines. Cancer cell lines with MET amplification (EBC-1, Hs 746T, SNU-5, MKN-45, NCI-H1993, and OE-33) and without MET amplification (H-460, HCT116) were treated for 96 hours with SAR125844 and proliferation rate was measured using CellTiterGlo or 14C-thymidine incorporation methods. C, proapoptotic activity of SAR125844 in the SNU-5 MET-amplified tumor cell line. SNU-5 cells were incubated with SAR125844 for 24, 48, or 72 hours and stained with Annexin V-Alexa488 and propidium iodide. Annexin V-positive cells were quantified by flow cytometry. D, SAR125844 inhibition of HGF-induced cell migration. Cell migration of PC-3 cells was initiated with 50 ng/mL HGF and SAR125844 was added for 20 hours. Inhibition of cell migration was quantified (left) and representative microscope field shown (right).
the experiment and radioactivity incorporation was measured using a Microbeta Wallac counter.

**Apoptosis assay**

SNU-5 cells were seeded in 6-well plates in complete medium and incubated with SAR125844 for 24, 48, or 72 hours before staining with Annexin V–Alexa488 and propidium iodide. Quantification of the Annexin V–positive cells was done using a EC800 Cytometer (Sony).

**Migration assay**

PC-3 cells were plated at a density of 60,000 cells per 200 μL of migration medium (DMEM without phenol red, 1% glutamine, 0.1% BSA) in the top chambers of BD Falcon Fluoroblock 24-well plates (BD Biosciences; 351158), in the presence of SAR125844. Fifty ng/mL HGF (R&D Systems; 294-HG) was added to the bottom wells. The plates were incubated at 37°C for 20 hours before cell scraping. The quantification of fluorescence was done from the bottom of the plates at 485 nm using a SpectraFluor Plus reader (Tecan).

**In vivo studies in tumor-bearing mice**

Procedures involving animal were conducted in accordance with the conditions established by the European Community (2010/63/EEI Directive) and approved by the Sanofi Animal Care and Use Committee. Severe combined immunodeficient (SCID) female mice, 8- to 10-week-old, bred at Charles River France, were at least 18 g at the start of treatment and had free access to food and sterile water. The human gastric carcinoma SNU-5 and Hs 746T cell lines, with 13 and 29 gene copies amplification of the MET gene, respectively (Supplementary Table S1) were implanted subcutaneously with 20 × 10⁶ cells per mouse and maintained by serial passages once every 3 to 4 weeks.

The pharmacokinetic, pharmacodynamics, and antitumor activity of SAR125844 was evaluated in mice bearing advanced stage (200–400 mm³) SNU-5 and Hs 746T xenografts. The pharmacokinetic parameters of SAR125844 were investigated in plasma and tumor tissue after a single intravenous administration at 45 and 20 mg/kg in SNU-5 and Hs 746T tumor–bearing mice, respectively. Blood and tumor samples were collected from three animals at 5, 15, and 30 minutes, and 1, 2, 4, 6, 8, 12, 24, 48, and 72 hours after SAR125844 treatment. SAR125844 concentrations were determined by liquid chromatography–tandem mass spectrometry. The pharmacodynamic impact of SAR125844 was documented in mice bearing tumors, randomized into experimental groups (group of 6 mice), and given a single intravenous dose of either vehicle or SAR125844 in the tail vein. SAR125844 solution was given at 5, 10, 20, and 45 mg/kg and SAR125844 nanosuspension was given at 53, 106, and 213 mg/kg doses. At designated time points posttreatment, mice were humanely euthanized, tumor and plasma collected for the SNU-5 tumors at 1, 4, 24, and 48 hours (solution formulation), and at 1, 2, 3, 4, and 7 days (nanosuspension formulation); for the Hs 746T tumors at 4, 24, and 48 hours (solution formulation) and 3, 4, 5, 6, 7 days (nanosuspension formulation). Resected tumors were processed in the Precells 24 bead beating homogenizer and endogenous levels of pMETh1234, pAKT647S, pMEK527/221, MET, and AKT evaluated using MSD assays (#K15126D-2, #K151100D-2, #K151CW-D-2) as recommended by the manufacturer. Statistical significance of the pharmacodynamic impact was determined by a Winer analysis and/or Dunnett test after a two-way ANOVA for each rank-transformed biomarker with $P \leq 0.05$ considered significant. Pharmacodynamic impact was also evaluated by immunohistochemistry of formalin-fixed, paraffin-embedded 4 μm sections using pMETH1234 antibody (Cell Signaling Technology #3077). Immunohistochemistry was conducted using Discovery XT autostainer (Ventana Medical Systems, Inc), with the DABMap kit (#760-124; Ventana Medical Systems, Inc.), according to the manufacturer’s instructions.

The antitumor activity of SAR125844 was investigated after administration of SAR125844 solution daily in SNU-5 bearing mice at 10, 20, and 45 mg/kg and every 2 days in Hs 746T tumor–bearing mice (at 5, 10, 20, and 45 mg/kg for SAR125844 solution and at 5, 11, 21, 53, 106, and 213 mg/kg for SAR125844 nanosuspension). Tumor volumes (in mm³, based on the following formula, volume = length (mm) × width² (mm²)/2), were measured twice weekly and body weight recorded every day. Endpoints collected were complete regressions (CR, regression below the palpable limit), partial regressions (PR, regression of 50% of the initial tumor volume), and the percentage of tumor regression (% of volume decrease posttreatment compared with pretreatment). Statistical significance was determined by a Dunnnett test versus vehicle after a two-way ANOVA with repeated measures performed separately on ranks of changes from baseline with $P < 0.05$ considered significant.

**Results**

SAR125844 is a potent, highly selective, and ATP-competitive inhibitor of wild-type and MET kinase mutants

SAR125844 (Fig. 1A) was identified following optimization of a triazolopyridazine scaffold for MET kinase inhibition and drug–like properties (32). In assays performed using purified proteins in a HTRF format, SAR125844 strongly inhibited the kinase activity of wild-type MET enzyme with IC₅₀ value of 4.2 nmol/L, as well as the H1094Y, Y1235D, M1250T, L1195V, and D1228H kinase domain mutants with IC₅₀ values of 0.22, 1.7, 6.5, 65, and 81 nmol/L, respectively. SAR125844 also inhibited the Y1230H mutant, although at a lower potency (IC₅₀ value of 204 nmol/L; Table 1). Additional kinetic studies indicated that SAR125844 is an ATP-competitive and reversible inhibitor (data not shown). The biochemical selectivity of SAR125844 was documented in a panel of 275 human kinases and against tubulin. SAR125844 was moderately active on RON, a close structural homolog of MET, with IC₅₀ value of approximately 740 nmol/L, suggesting that SAR125844 was highly (>100-fold) selective for the MET kinase over RON. Minimal inhibitory activity was identified on 5 additional kinases with IC₅₀ values below 300 nmol/L, including TRKA/NTRK1 (39 nmol/L), TRKB/NTRK2 (280 nmol/L), PDGFRα-V561D (55 nmol/L), AXL (87 nmol/L), and MER (105 nmol/L; Table 1). Biochemical activities on Aurora A and Aurora B were detected with IC₅₀ values of 320 and 820 nmol/L, respectively, but these activities did not translate into cellular activity as demonstrated by the lack of antiproliferative activity in tumor cell lines without MET gene amplification (Fig. 1B and Supplementary Table S1). No activity on tubulin was detected up to 25 μmol/L of SAR125844.

SAR125844 inhibits MET kinase activity in tumor cell lines

The effect of SAR125844 on MET kinase autophosphorylation was evaluated in the Hs 746T, SNU-5, and MKN-45 gastric cancer cell lines with MET gene amplification with 29, 13, and 21 gene copies, respectively.
copies, respectively. SAR125844 demonstrated a potent inhibition of MET phosphorylation with IC50 values of 1.4, 3.9 and 5.1 nmol/L in Hs 746T, SNU-5, and MKN-45 cell lines, respectively (Table 2). The effect of SAR125844 on MET kinase mutants was evaluated in HEK-293T cells transiently expressing wild-type MET, and the Y1235D, M1250T, L1195V, Y1230H and D1228H MET kinase domain mutants. SAR125844 inhibited the phosphorylation of wild-type MET, M1250T, Y1235D, and L1195V MET mutants, with IC50 values of 8.8, 30, 179, and 591 nmol/L, respectively. No activity of SAR125844 was observed in cells expressing Y1230H and D1228H MET mutants. SAR125844 selectivity against AXL and NTRK1 kinases was further documented in cell-based assays. SAR125844 moderately inhibited the autophosphorylation of AXL in transfected MEFs, with an IC50 value of 110 nmol/L, and the proliferation of the KM12 cell line expressing the TPM–NTRK1 fusion protein (34), with an IC50 value of 1.4 µmol/L (Table 1).

SAR125844 selectively inhibits proliferation, promotes apoptosis in cell lines with MET gene amplification, and inhibits HGF-mediated tumor cell migration

The activity of SAR125844 on cell proliferation was evaluated in a panel of 31 tumor cell lines, including cells with MET gene amplification, cells with HGF-mediated activation and MET/ HGF-independent cell lines (Supplementary Table S1, with few representative examples shown in Fig. 1B). SAR125844 inhibited cell proliferation in all cell lines with MET gene amplification (copy number range of 9–32), with IC50 values in the nanomolar range (1–7 nmol/L), with the exception of the lung cancer cell line NCI-H1573. No impact was observed in wild-type cells without MET gene amplification. However, when some of these cells were activated by exogenous addition of HGF, SAR125844 was able to inhibit their HGF-induced proliferation (Supplementary Table S1). SAR125844 was active in the U-87 MG glioblastoma cell line with an HGF-mediated autocrine activation, with a maximum inhibition of 50% and a relative IC50 value of 25 nmol/L. SAR125844 was active in the MNNG/HOS osteosarcoma cell line expressing the TPR-MET fusion protein, with an IC50 value of 14 nmol/L. SAR125844 was inactive in the autocrine KP-4 pancreatic cell line and in the NCI-H596 cell line harboring a MET exon 14 deletion.

Proliferation was also used as a readout to evaluate the duration of action of SAR125844 in the MET-amplified Hs 746T gastric tumor cell line. Cells were exposed to SAR125844 for various times (1, 4, 8, 24, 48, and 72 hours), washed out and grown in the absence of SAR125844 for a total of 72 hours before evaluating the impact on cell proliferation. SAR125844 inhibited proliferation with a short exposure of 1 hour (IC50 value of 55 nmol/L), with the impact being more important with longer exposures (IC50 value of 3 nmol/L, Supplementary Table S2). This suggested that a short exposure of MET-amplified cells to SAR125844 was sufficient to induce potent and durable inhibition of cell proliferation.

The mechanism of action of SAR125844 was further characterized. SAR125844 induced cell-cycle arrest in G1 phase (data not shown) in the two MET-amplified Hs 746T and MKN-45 gastric cancer cell lines, and induced massive apoptosis in SNU-5 cells at 48 and 72 hours, with a relative EC50 value of 6 nmol/L and a plateau of 60% at 72 hours (Fig. 1C). Besides, as the MET pathway regulates cell migration and invasion, the impact of SAR125844 on HGF-induced cell migration was evaluated with the PC-3 prostate cancer cell line. SAR125844 inhibited cell migration with an IC50 value of 26 nmol/L (Fig. 1D).

Taken together, our data suggested that SAR125844 was highly potent and selective for the MET kinase in cell-based assays.

SAR125844 exhibits a high tumor exposure and inhibits MET phosphorylation in gastric tumor–bearing mice

Pharmacokinetic and pharmacodynamic studies were conducted in mice bearing xenograft tumors of Hs 746T and SNU-5 gastric cancer cell lines with MET gene amplification. Plasma and tumor pharmacokinetic parameters of SAR125844 were investigated after a single intravenous administration. A large volume of distribution and a moderate total plasma clearance (35% and 59% of mouse hepatic blood flow, respectively) were documented. SAR125844 exposure was higher in tumor versus plasma, with a tumor to plasma AUC ratio equal to 3.1 in SNU-5 and 8.1 in Hs 746T models (Supplementary Table S3), and with a very long elimination half-life in tumors (Fig. 2A and D). Pharmacodynamic analysis in SNU-5 tumors demonstrated a time-dependent reduction of MET kinase activity, and inhibition of downstream PI3K and MAPK pathways. Following administration of 45 mg/kg SAR125844, pMET Y1349, pAKT S473, and MAPK phosphorylation in gastric tumor extracts revealed that MET kinase inhibition was complete (99%) within 1 hour, maintained for 4 hours, and was partial (59%) at 24 hours (Fig. 2A–C). The pharmacodynamic impact of SAR125844 was further confirmed.

### Table 1. Enzymatic and cell-based kinase inhibition profile of SAR125844

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Biochemical IC50 (nmol/L)</th>
<th>95% CI</th>
<th>Cellular IC50 (nmol/L)</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>MET</td>
<td>Wild-type</td>
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<td></td>
<td>H1094Y</td>
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<td>0.12–0.42</td>
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<tr>
<td></td>
<td>Y1235D</td>
<td>1.7</td>
<td>1.1–2.8</td>
<td>179</td>
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<tr>
<td></td>
<td>M1250T</td>
<td>6.5</td>
<td>4.5–9.5</td>
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<td></td>
<td>L1195V</td>
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<td>RON/MSTIR</td>
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<td>nd</td>
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<td>PDGFRα/β-V561Dc</td>
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<td>AXLc</td>
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<td>nd</td>
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<td></td>
<td>MER2</td>
<td>105</td>
<td>nd</td>
<td>nd</td>
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</table>

Abbreviations: CI, confidence interval; nd, not defined.

aBiological selectivity profile against a panel of 275 kinases. IC50 concentration required for 50% target inhibition.

bTested on the Millipore panel.

cKinases with IC50 values below 300 nmol/L.

dProQinase cellular assay.

eKM12 (TPM–NTRK1 fusion) proliferation assay.

fProQinase cellular assay (ProQinase).

### Table 2. Effect of SAR125844 on MET autophosphorylation in gastric cancer cell lines

<table>
<thead>
<tr>
<th>Gastric cancer cell line</th>
<th>MET gene copy</th>
<th>IC50 (nmol/L)</th>
<th>95% CI</th>
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<tr>
<td>Hs 746T</td>
<td>29</td>
<td>1.4</td>
<td>1.2–1.6</td>
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<td>SNU-5</td>
<td>13</td>
<td>3.9</td>
<td>1.1–13.7</td>
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<td>MKN-45</td>
<td>21</td>
<td>5.1</td>
<td>3.2–8.0</td>
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</table>

Abbreviation: CI, confidence interval.

*Determined by CGH analysis for Hs 746T and SNU-5 and from ref. 49 for MKN-45.

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by immunohistochemical analysis with pMETY1234/1235 staining in tumor tissue (Fig. 2B). Expression of pMETY1234/1235 was completely abolished at 4 hours with administration of 45 mg/kg SAR125844 and restored to 45% at 15 hours and 65% at 24 hours. The complete inhibition of MET activity was associated with an important inhibition of PI3K and MAPK pathways (reduction of 83% of pAKTS473 and 77% of pMEKS217/221 at 4 hours; Fig. 2C). When the MET inhibition was partially released at 24 hours, a paradoxical overactivation of downstream MAPK and PI3K pathways was observed, probably
reflecting the addiction of these pathways to MET kinase activity in this model. This overactivation was transient and was back to normal level at 72 hours (Fig. 2C). A dose-dependent pharmacodynamic impact on MET, PI3K, and MAPK pathways was observed at 20 and 10 mg/kg of SAR125844. When SAR125844 was compared with JNJ-38877605 and PF-04217903, two oral selective MET kinase inhibitors, in the SNU-5 model, the pharmacodynamic impact of the oral inhibitors was similar to the one observed with SAR125844 (data not shown) with the transient paradoxical overactivation of MAPK and PI3K pathways also observed with JNJ-38877605 and PF-04217903. SAR125844 had a similar, dose- and time-dependent pharmacodynamic impact in the Hs 746T tumors (Fig. 2D and E). However, the Hs 746T tumors were highly sensitive to SAR125844, where similar SAR125844 tumor exposure (Supplementary Table S2) led to a longer (24 hours) and more potent (80%) inhibition of MET kinase activity associated with significant inhibition (60%–80%) of downstream pathways (Fig. 2E). A partial MET inhibition was maintained for 48 hours, and unlike what was observed in the SNU5 model, there was no evidence of overactivation of downstream pathways. Interestingly, in both SNU-5 and Hs 746T tumor models, significant MET kinase inhibition was observed even when no plasma drug concentration was detected (Fig. 2A and D), therefore no direct correlation between plasma SAR125844 concentration and MET inhibition was established. The high potency of SAR125844, even with short exposure, and higher tumor versus plasma exposure, might contribute to this long-lasting inhibition, and was not observed with the first generation selective MET inhibitors such as INJ-38877605 and PF-04217903 (Supplementary Table S3). Taken together, our results demonstrate that SAR125844 is able to completely inhibit MET kinase in two MET-amplified tumor models, with strong impact on downstream MAPK and PI3K pathways.

SAR125844 displays potent antitumor activity in gastric tumors with MET gene amplification

The relationship between MET kinase inhibition by SAR125844 and tumor growth inhibition was evaluated with repeated dosing efficacy studies. Repeated intravenous administration of SAR125844 in SNU-5 and Hs 746T tumor–bearing mice was well tolerated with no treatment-related relevant body weight loss at all doses tested. Daily dosing in SNU-5 tumor-bearing mice of SAR125844 at 45 mg/kg resulted in complete and almost complete tumor regression in 3 of 8 mice and 5 of 8 mice, respectively. Partial tumor regression were observed in 8 of 8 mice at 20 mg/kg and tumor stasis at 10 mg/kg (Fig. 3A). As significant MET kinase inhibition was maintained in Hs 746T tumor for 48 hours, efficacy studies were performed in this model with SAR125844 administered in an every-2-days regimen. Every-2-day dosing in Hs 746T tumor–bearing mice of SAR125844 at 45 mg/kg resulted in complete and partial (85%) tumor regression in 2 of 7 and 5 of 7 mice, respectively (Fig. 3B). Partial tumor regression were observed in 8 of 8 mice at 20 mg/kg and in 5 of 7 mice at 10 mg/kg every 2 days.

The antitumor activity of SAR125844 was further confirmed using a nanosuspension formulation that allowed higher strength administrations of SAR125844 up to 213 mg/kg with no relevant treatment-related toxic effects. The antitumor activity of the nanosuspension and the solution formulations were compared with an every-2-days regimen in the Hs 746T model. Complete tumor regression were observed in 7 of 8 of mice at 53, 106, and 213 mg/kg, and partial regression were observed at
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Figure 4. SAR125844 nanoformulation administration results in long duration pharmacodynamic impact and tumor regression in MET-amplified gastric tumor models. A, mice bearing established subcutaneous tumors derived from Hs 746T gastric cancer cell line were administered every 2 days (from day 17 to 23) the doses of a colloidal nanosuspension drug delivery system of SAR125844 or vehicle. Each data point represents the mean ± SEM of the calculated tumor volumes observed in experimental groups including 8 mice. Statistical significance was determined by a Dunnett test versus vehicle after a two-way ANOVA with repeated measures performed separately for each compounds on ranks of changes from baseline with \( P < 0.05 \) considered significant (asterisks indicate statistically significant differences between treated and control experimental groups; ***, \( P < 0.001 \)). B and C, mice bearing established subcutaneous SNU-5 or Hs 746T gastric cancer tumors were administered with a single high strength dose (53, 106, and 213 mg/kg) of a colloidal nanosuspension of SAR125844 by intravenous route. B, impact on tumor mass in mice bearing gastric cancer tumors after single high doses of SAR125844 nanosuspension evaluated day 1 (24 hours), 2 (48 hours), 3 (72 hours), 4 (96 hours), 5 (120 hours), 6 (144 hours), and 7 (168 hours) postadministration. C, correlation of pharmacodynamic impact on MET kinase activity and impact on tumor mass in mice bearing SNU-5 gastric cancer tumors after single high dose of SAR125844 in nanosuspension.

11 and 21 mg/kg, similar to what was observed with the solution formulation (compare Fig. 3B with 4A). Then, we evaluated the impact of single high strength administration of the SAR125844 nanosuspension on MET kinase activity and on tumor mass. In the SNU-5 model, more than 80% MET inhibition was observed at all doses of SAR125844 and this inhibition was maintained for 96 hours at 213 mg/kg, 72 hours at 106 mg/kg, and 48 hours at 53 mg/kg (Fig. 4C). A MET inhibition greater than 80% for 96 hours resulted in a near complete tumor regression (93% reduction) starting at day 4 and maintained at day 7, while the same level of inhibition lasting for 72 hours resulted in a partial regression (62% reduction; Fig. 4B and C). In the Hs 746T model, complete or near-complete regression (92%–99%) was achieved 3 days after a single administration and maintained for 7 days at all dose levels (Fig. 4B). Taken together, our results indicated that SAR125844 was able to maintain a long duration of MET inhibition up to 96 hours, and induce tumor regression in two MET-amplified gastric tumor models after a single high strength administration without any relevant impact on body weight.

**Discussion**

Several selective kinase inhibitors of MET have been identified and are still in the early stages of clinical development. Here, we report the preclinical activity of SAR125844, a potent, selective, ATP-competitive MET kinase inhibitor, with single-digit nanomolar antiproliferative activity in MET-amplified cell lines and able to promote tumor regression in MET-amplified xenograft models.

SAR125844 is highly selective for MET over its close homolog RON *in vitro*. Inhibition of five additional kinases, NTRK1, NTRK2, PDGFRα-V561D, AXL, and MER, was identified and
the impact in cell-based assays was documented for the most sensitive ones, AXL and NTRK1. SAR125844 inhibited autophosphorylation of AXL and cell proliferation of TPM-NTRK1-overexpressing Km12 cell line with IC\textsubscript{50} values of 110 and 1,400 nmol/L, respectively, indicating a 30- and 500-fold selectivity index for AXL and NTRK1 in cell-based assays. The selectivity profile of SAR125844 was further confirmed in cell lines with a single-digit nanomolar antiproliferative activity in MET-addicted cell lines and a complete lack of impact in cells not addicted to the MET pathway. This is in contrast to ARQ197 that has equal antiproliferative activity on MET-addicted and MET-independent tumor cell lines (35, 36). This is also in contrast to crizotinib, which has in addition to its potent activity on MET and ALK-driven tumor cell lines, micromolar antiproliferative activity in cell lines not driven by MET or ALK alterations (37).

Amplification of the MET gene was identified in several cancers and is thought to be an oncogenic driver in subsets of patients with gastric, esophageal, and lung cancers, and selective MET kinase inhibitors are expected to provide a clinical benefit in these patients. In the MET-amplified tumor cell lines tested, SAR125844 was highly active in 6 of 7 cell lines, with proapoptotic activity and single-digit nanomolar IC\textsubscript{50} values in cell proliferation assay. As previously reported (38), the NCI-H1573 cell line was insensitive to MET kinase inhibition, probably due to the constitutive activation of the MAPK pathway downstream of MET mediated by a KRAS mutation (Supplementary Table S1). The antiproliferative effect of SAR125844 was limited to cell lines with MET amplification and to cells with activation of the MET pathway upon HGF binding. No impact was observed in cell lines without MET amplification, suggesting that amplification of MET or high phospho-MET expression could be potential predictive markers for SAR125844 activity in patients. We conducted a prospective molecular epidemiology study to determine the prevalence of MET gene amplification in a small cohort of advanced gastric patients of Japanese and Korean origin and have found 5 of 32 (15.6%) patients with >10% of tumor cells with MET gene amplification (copies >4 and a MET/CEP7 ratio ≥2; ref. 39).

Daily or every-2-days administration of SAR125844 led to a potent and sustained inhibition of MET autophosphorylation and of downstream PI3K and MAPK pathways and tumor regression in MET-amplified gastric xenograft models without any sign of toxicity or body weight loss even at high doses tested. A 75% to 80% inhibition of MET and downstream pathways was maintained for 24 hours in the most sensitive Hs 746T model, and for 4 hours in the SNU-5 model. These data suggest that, due to the strong addiction to the MET pathway, a robust pathway inhibition for a fraction of the dosing interval was necessary to achieve tumor regression. Because of its intravenous route of administration, a weekly schedule of SAR125844 is anticipated in patients. Therefore, we evaluated a single administration of high doses of SAR125844 in the SNU-5 and Hs 746T models, and observed long-lasting MET kinase inhibition up to 4 days, consistent with the potent impact on cell proliferation observed with a short 1 hour exposure of SAR125844 in the washout experiments. The higher tumor exposure versus plasma of SAR125844 was also observed with some type I MET inhibitors such as EMD1214063 but not with the first generation selective MET inhibitors such as JNJ-38877605 and PF-04217903 (Supplementary Table S3). A weekly regimen will allow an intermittent administration of high doses of SAR125844 in MET-amplified cancer patients and could potentially prevent the emergence of resistance mutations. Two phase I clinical studies of SAR125844 monotherapy administered on a weekly schedule by intravenous infusion are ongoing in Caucasian (NCI01391533) and Asian (NCI01657214) patients with advanced solid tumors. Preliminary results of NCT01391533 study in solid tumor patients indicated a favorable tolerance profile and promising preliminary evidence of antitumor activity in a patient with lung adenocarcinoma with MET gene amplification (40). Safety and antitumor activity is further evaluated in a preselected cohort of patients with MET amplification.

Over 30 missense MET mutations were reported and the oncogenic potential of some of them documented (41). Although not yet documented in cancer patients but described in in vitro cell systems (42, 43), mutations will probably emerge due to an acquired resistance to MET kinase inhibitors. We characterized the activity of SAR125844 on 6 MET mutant variants, including H1094Y, L1195V, Y1230H variant, responsible of resistance to type I MET selective inhibitors, and M1250T and D1228H variants that have equivalent mutations in RET and KIT kinase domains. The Y1235D variant was described as frequent in head and neck cancer (44, 45) but a recent study in oropharyngeal tumor samples challenged this conclusion (46). SAR125844 was active at nanomolar concentration on all 6 enzymatic variants and active on cells expressing M1250T, Y1235D and L1195V mutants at IC\textsubscript{50} values of 30, 179, and 591 nmol/L. In contrast, SAR125844 was inactive on cell lines expressing D1228H and Y1230H mutants, despite biochemical activity of 81 and 204 nmol/L, respectively, suggesting that an affinity of 50 nmol/L or lower is necessary to translate into significant MET inhibition in cell lines. Overall, the activity of SAR125844 on these selected MET mutants is equivalent to the one observed with EMD1214063 (47), and suggests that highly selective type I MET inhibitors might not be active on MET mutants such as Y1230H or D1228H compared with type II nonselective MET inhibitors. Interestingly, in contrast to somatic mutations, germline MET mutations were highly predictive of a response to foretinib in patients with papillary renal cell carcinoma (48). As MET somatic mutations are not clustered in any hotspot region and have low prevalence in patients, the prospective selection of a specific patient subpopulation with a MET variant would be operationally challenging.

In conclusion, our data demonstrated that SAR125844 is a potent and selective MET kinase inhibitor, able to induce tumor regressions in MET-amplified tumor models, with a single high strength dose administration, and with a favorable preclinical toxicity profile, supporting its clinical development in patients with MET-amplified tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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