Foretinib Is Effective Therapy for Metastatic Sonic Hedgehog Medulloblastoma

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Abstract

Medulloblastoma is the most common malignant pediatric brain tumor, with metastases present at diagnosis conferring a poor prognosis. Mechanisms of dissemination are poorly understood and metastatic lesions are genetically divergent from the matched primary tumor. Effective and less toxic therapies that target both compartments have yet to be identified. Here, we report that the analysis of several large non-overlapping cohorts of patients with medulloblastoma reveals MET kinase as a marker of sonic hedgehog (SHH)–driven medulloblastoma. Immunohistochemical analysis of phosphorylated, active MET kinase in an independent patient cohort confirmed its correlation with increased tumor relapse and poor survival, suggesting that patients with SHH medulloblastoma may benefit from MET-targeted therapy. In support of this hypothesis, we found that the approved MET inhibitor foretinib could suppress MET activation, decrease tumor cell proliferation, and induce apoptosis in SHH medulloblastomas in vitro and in vivo. Foretinib penetrated the blood–brain barrier and was effective in both the primary and metastatic tumor compartments. In established mouse xenograft or transgenic models of metastatic SHH medulloblastoma, foretinib administration reduced the growth of the primary tumor, decreased the incidence of metastases, and increased host survival. Taken together, our results provide a strong rationale to clinically evaluate foretinib as an effective therapy for patients with SHH-driven medulloblastoma. Cancer Res; 75(1); 134–46. ©2014 AACR.

Introduction

Medulloblastoma, the most common malignant brain tumor in childhood, has a high tendency to disseminate through the cerebrospinal fluid to the brain and the spinal cord leptomeninges. Dissemination is a known factor of poor survival and occurs in one third of the children at the time of diagnosis and in two thirds by the time of relapse. Affected children are treated with craniospinal radiation and high-dose chemotherapy but the majority of survivors suffer from severe neurocognitive deficits induced by the deleterious effects of treatment on the developing nervous system (1). The discovery of novel and less toxic therapies has been hampered by the poor understanding of the mechanisms of dissemination, and by the failure to account for the genetic divergence between metastatic lesions and their matched primary tumor. Recent studies have shed light into the candidate genes that drive leptomeningeal dissemination in medulloblastoma (2, 3), but therapies that target both the primary and the metastatic compartment have not been identified.

The hepatocyte growth factor (HGF)/cMET pathway is essential for cell proliferation and migration during embryogenesis (4) and, in the central nervous system, it plays a critical role in cerebellar development (5). Aberrant cMET signaling is known to be involved in tumor growth and metastatic behavior of several human cancers (6, 7). The transmembrane receptor cMET is activated through phosphorylation of tyrosine residues upon binding of its ligand HGF, which triggers multiple downstream effector cascades including MAPK and PI3K/AKT that function in various cellular processes including cell proliferation, cell survival, migration, and invasion (8). In medulloblastoma, cMET activation is associated with tumor growth and anaplastic histology (9). cMET signaling is deregulated in medulloblastoma through multiple, independent molecular mechanisms including epigenetic silencing of an upstream inhibitor, the serine protease inhibitor Kunitz-type 2 (SPIN72; refs. 10, 11). Previous studies have shown that cMET inhibition can effectively decrease medulloblastoma cell migration and invasion (12).
Foretinib is an orally available multikinase inhibitor that targets cMET with high affinity (IC$_{50}$ = 0.4 nmol/L; ref. 13). Foretinib has demonstrated antitumor activity in preclinical models of different tumor types (14–16) and clinical trials are currently ongoing to determine its efficacy in various solid, non-central nervous system tumors (17, 18). To date, the ability of foretinib to penetrate the brain is unknown.

Foretinib also targets other tyrosine kinases with lower affinity, including the platelet-derived growth factor receptor beta (PDGFR$\beta$) with an IC$_{50}$ of 9.6 nmol/L. Interestingly, PDGFR$\beta$ is known to be overexpressed in metastatic medulloblastoma (19, 20), and targeting the receptor reduces proliferation and migration of medulloblastoma cell lines (21). Activation of PDGFR$\beta$ occurs through a similar mechanism to cMET receptor activation, in which ligand binding (PDGF-BB) induces receptor autophosphorylation and activates downstream signaling via MAPK and AKT (22).

Therefore, we sought to establish the subgroup-specific role of cMET and PDGFR$\beta$ in medulloblastoma, and to test the efficacy of foretinib to cross the blood–brain barrier and to target those pathways, both in the primary and in the metastatic compartments.

**Materials and Methods**

**Tumor material and patient characteristics**

All tissues and clinicopathologic information were serially collected in accordance with Institutional Review Boards from contributing institutions. Nucleic acid extractions were carried out as previously described (23).

**Expression profiling and molecular subgrouping**

Expression of candidate genes was assessed using the R2 software in independent gene expression cohorts (24–32). Expression of reported intermediates of MET signaling was visualized using heatmaps (7). Associations between gene expression and subgroup affiliation were evaluated using one-way ANOVA. P values < 0.05 were considered to be statistically significant.

**Analysis of somatic copy-number alterations**

Somatic copy-number alterations were assessed on the Affymetrix SNP 6.0 array platform in 1,239 cases. Raw copy-number estimates were obtained in dChip, followed by circular binary segmentation (CBS) in R as previously described (27).

**Cell lines and animal models**

Human medulloblastoma cell lines (Daoy, ONS76 and D425) were kindly provided by Dr. Annie Huang, Hospital for Sick Children, in Toronto. All cell lines were authenticated and tested by PCR. Daoy-GFP/Luciferase cells were generated as described previously (33). Athymic nude mice were obtained from Charles River Laboratory. Pchc$^{+/+}$/SB11/T2Onc mice (2) were generously provided by Dr. Michael Taylor, Hospital for Sick Children, Toronto, Canada.

**Cell culture assays for cMET and PDGFR$\beta$ signaling**

Foretinib was purchased from Selleck Chemicals, dissolved in DMSO (Sigma), and stored at −20°C. Cells were serum starved for 24 hours in media with 0.1% (Daoy and ONS76) and 2% FBS (D425), pretreated with increasing concentrations of foretinib or 1% v/v DMSO for 2 hours, and stimulated with human recombinant HGF (20 ng/mL, Sigma-Aldrich) for 20 minutes or human PDGF-BB (20 ng/mL; Cell Signaling) for 10 minutes.

**Migration and invasion assays**

The protocols for migration and invasion assays were as described previously (34).

For the radial migration assays, medulloblastoma cells were seeded and allowed to migrate for 24 hours in starved media (0.1% FBS) containing HGF (50 ng/mL) or PDGF-BB (50 ng/mL), in the presence or absence of foretinib. The radius of the migrating cells was measured and compared with the initial radius using a Leica Fluorescent Stereoscope (2.5× magnification).

The invasion assays were performed using Matrigel Invasion Chambers (8 μm pore size; BD Biosciences). Daoy and ON576 cells were incubated for 16 hours with starved media containing dilutions of foretinib. The number of cells per 6 random fields was determined (>10 magnification) using Volocity software (Perkin Elmer).

**Immunoblotting**

The following antibodies from Cell Signaling were used: cMET (1:1,000), phospho-cMET (1:1,000), PDGFR$\beta$ (1:500), phospho-PDGFR$\beta$ (1:500), AKT (1:1,000), phospho-AKT (1:2,000), p44/42 MAPK (1:1,000), phospho-p44/42 MAPK (1:2,000), PARP (1:1,000), β-actin (1:10,000), anti-rabbit IgG conjugated to horseradish peroxidase (1:5,000), and anti-mouse IgG conjugated to horseradish peroxidase (1:5,000). Western blot analysis and quantification were performed using the Fluorchem Q Imaging System (ProteinSimple).

**Cell proliferation assays**

Medulloblastoma cells were treated with different concentrations of foretinib or DMSO, and cell viability was determined for the indicated time points by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) absorbance at 490 nm (CellTiter 96 Aqueous One Solution Reagent; Promega).

**Active caspase assays**

The activity of caspases 3 and 7 was measured using the ApoONE Homogeneous Caspase-3/7 Assay (Promega) after 12 hours of incubation.

**Foretinib pharmacokinetic studies**

Detection and quantification of foretinib were performed using a high-performance LC/MS/MS method (Agilent 1290 HPLC Agilent Technologies/QTRAP 5500 AB SCIEX).

Images of foretinib distribution in various mouse organs were acquired using a matrix-assisted laser desorption/ionization MALDI) time-of-flight tandem mass spectrometer (AB SCIEX TOF/TOF 5800 System; AB SCIEX).

**Medulloblastoma xenografts and transgenic mouse models**

All mouse studies were approved and performed in accordance with the policies and regulations of the Institutional Animal Care and Use Committee of the University of Toronto and the Hospital for Sick Children, in Toronto.

Mouse subcutaneous xenografts (Daoy and ONS76) were established in athymic nude mice (Charles River Laboratories). Intracranial xenografts of disseminated medulloblastoma were...
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established by injecting Daoy cells into the fourth ventricle of athymic nude mice. Tumor growth and the presence of metastases were evaluated by weekly bioluminescence imaging using the IVIS Spectrum Optical In-vivo Imaging System (Caliper Life Sciences).

Osmotic pumps (Alzet; model 2004) were implanted in Pchktm−/− mice with Sleeping Beauty (SB) transposition (Pchktm−/−/SB11/T2Onc) at postnatal days 30 to 35. Pumps loaded with foretinib (6 mg/kg) infused the drug into the cerebrospinal fluid (right lateral ventricle) for 28 days at a rate of 0.25 μL/hour.

Immunohistochemistry

The protocol for immunohistochemistry was performed as previously described (35). The following antibodies were used: Ki-67 (1:1,000; Novus), cleaved caspase 3 (1:800; Cell Signaling), phospho-cMET (1:100; Cell Signaling), and phospho-PDGFRβ (1:200; Abcam).

Statistical analysis

Survival curves were generated using the Kaplan–Meier estimate and a log-rank test. The comparison between binary and categorical patient characteristics was performed using the two-sided Fisher exact test. To analyze contiguous variables, the Mann–Whitney U test was used.

Results

cMET and PDGFRβ are highly expressed in SHH medulloblastomas

We evaluated the expression of cMET and PDGFRβ in a discovery cohort of primary medulloblastomas from Boston (n = 199; ref. 26). cMET expression was highly upregulated in most SHH medulloblastomas and in a subset of group 3 tumors (Fig. 1A). PDGFRβ was expressed across all medulloblastoma subgroups, with higher levels of expression in SHH tumors (Fig. 1B). We confirmed our results using a multicenter validation cohort of 439 cases profiled on the Affymetrix 133PLUS 2.0 arrays obtained from the German Cancer Research Centre, Heidelberg (validation cohort; refs. 24, 25, 31, 32, M. Kool and S.M. Pfister, unpublished data; Fig. 1C and D).

To identify molecular markers of cMET pathway activation in SHH medulloblastomas, we interrogated three independent data-sets using a group of genes known to activate cMET signaling (7, 8). In addition to cMET, GAB1 expression was highly and specifically upregulated in SHH-driven medulloblastomas, across the three nonoverlapping cohorts, when compared with normal cerebellum and with non-SHH medulloblastomas (Fig. 1E-I).

To investigate possible mechanisms leading to cMET and PDGFRβ overexpression, we examined the subgroup-specific copy number aberrations (CNA) encompassing the MET loci (7q31.2) and the PDGFRβ loci (5q31), in a large cohort of 1,239 medulloblastomas (27). There were no focal gains or amplifications of cMET (Supplementary Fig. S1A) and very infrequent copy-number gains affecting the PDGFRβ loci (Supplementary Fig. S1B).

Identification of biologic pathways and processes associated with high cMET and low cMET SHH medulloblastomas

We performed gene set enrichment analysis between tumors with the highest (n = 13) and the lowest (n = 13) cMET expression across a series of 51 primary SHH medulloblastomas (27). Gene sets were compiled from Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), the National Cancer Institute (NCI), Protein Families (PFAM), and Biocarta pathway databases. To visualize significant gene sets (FDR < 0.25; P < 0.01) as interaction networks, we used Cytoscape and Enrichment Map (Supplementary Fig. S2). High cMET SHH medulloblastomas were characterized by gene sets involved in organ development and morphogenesis, cell migration, cell cycle and DNA repair, T-cell differentiation, transcription, and mitochondrial function. Low cMET SHH medulloblastomas were defined by numerous networks including neural development, neurotransmission, amino acid and nucleotide metabolism, ribonucleotide biosynthesis, smooth muscle contraction, and Ras-GTPase activity.

High p-cMET levels correlate with recurrence and poorer survival in SHH medulloblastomas

We stained medulloblastoma tissue microarrays comprised of an independent cohort of 385 patients for the activated cMET receptor or phosphorylated cMET (p-cMET). Eighty percent (104/128) of SHH tumors and approximately 25% (16/62) of group 3 tumors showed high p-cMET staining (Fig. 2A). High p-cMET was associated with older age at diagnosis (Supplementary Fig. S3A and S3B) and desmoplastic histology (Supplementary Fig. S3C and S3D), most likely due to a subgroup-specific enrichment with SHH patients. There was a significant correlation between high p-cMET and an increased 5-year rate of recurrence across all subgroups of pediatric medulloblastomas (P = 0.041; Fig. 2B) and, particularly, across pediatric SHH tumors (P = 0.011; Fig. 2C). There was no association of p-cMET expression and the presence of metastases or TP53 mutational status in SHH tumors (Supplementary Fig. S3E and S3F).

High p-cMET expression in SHH tumors was associated with a significantly shorter progression-free survival (P = 0.007; Fig. 2D), particularly in the pediatric cohort (P = 0.002; Fig. 2E). A trend toward a worse overall survival was also observed in these patients (Fig. 2F and G). Interestingly, considering the metastatic status within SHH medulloblastomas, the prognostic impact of high p-cMET expression was still observed. High p-cMET correlated (P = 0.022) with a poor outcome in
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Figure 2. cMET pathway activation identifies subgroups of medulloblastoma with distinct clinical outcomes. A, immunopositivity for activated cMET (p-cMET) is a hallmark feature of SHH medulloblastomas identified by immunohistochemistry of medulloblastoma tissue microarrays in a cohort of 385 patients. Representative photographs of tumors with high p-cMET and low p-cMET staining are shown. Scale bar, 100 μm. B and C, in pediatric medulloblastomas, high p-cMET correlates with an increased 5-year recurrence rate across all subgroups (P = 0.041; B) and in the SHH subgroup (P = 0.011; C). D–I, Kaplan–Meier survival curves displaying progression-free survival (PFS) and overall survival (OS) in SHH medulloblastomas according to age (D–G) and the presence of leptomeningeal dissemination at diagnosis (H and I). M0, nonmetastatic tumors; M+, metastatic tumors.

Thus, we conclude that activated cMET defines distinct prognostic patient cohorts with higher recurrence rate and poorer prognosis in SHH subgroup of medulloblastomas.

Foretinib inhibits cMET and PDGFRβ pathway activity
On the basis of the elevated expression levels of cMET and PDGFRβ in SHH and group 3 medulloblastomas, we sought to evaluate the antitumoral effect of foretinib against medulloblastoma cell lines representative of these two subgroups. Daoy, ONS76, and D425 cells have transcriptional and cytogenetic features that suggest they are originally derived from

nonmetastatic SHH patients (Fig. 2H and Supplementary Fig. S4A). A trend toward a worse survival was observed in patients with high p-cMET and leptomeningeal dissemination at diagnosis (Fig. 2I and Supplementary Fig. S4B). In the pediatric cohort, high p-cMET was correlated (P = 0.014) with a poor progression-free survival (Supplementary Fig. S4C and S4D). Survival differences could not be determined in adult patients with SHH medulloblastoma according to p-cMET status (Supplementary Fig. S4E and S4F). In group 3 medulloblastomas, expression of p-cMET had no prognostic relevance and did not correlate with metastatic status.
SHH (Daoy and ONS76) and group 3 (D425) tumors (36). Furthermore, they express different levels of the tyrosine kinase receptors cMET and PDGFRβ (Supplementary Fig. S5A). First, we evaluated the ability of foretinib to inhibit the cMET and the PDGFRβ pathway activity in the context of HGF or PDGF-BB stimulation. Foretinib potently inhibited the HGF-induced cMET pathway activation, as evidenced by the suppression in cMET phosphorylation and also by the decrease in the phosphorylation of downstream effectors AKT and MAPK (Fig. 3A and B). The inhibitory effect of foretinib in PDGFR-β–induced PDGFRβ pathway activation was moderate (Supplementary Fig. S5B and S5C). Furthermore, a significant antiproliferative effect (Fig. 3C; Supplementary Fig. S6A and S6B) and an induction of apoptosis (Fig. 3D and E; Supplementary Fig. S6C and S6D) were observed in foretinib-treated medulloblastoma cells. Foretinib potently inhibited HGF-mediated migration (Fig. 3F and G; Supplementary Fig. S6E and S6F) and invasion (Fig. 3H and Supplementary Fig. S6G) of Daoy and ONS76 cells for all drug concentrations. In contrast, a significant reduction in migration and invasion mediated by PDGF-BB was only seen with 2.5 μmol/L of foretinib (Supplementary Fig. S6H and S6I).

We then assessed if cMET receptor knockdown by stable shRNA expression would phenocopy the functional effects of foretinib treatment. cMET shRNA 2 was able to knockdown over 70% of cMET expression in both Daoy (Fig. 3I) and ONS76 (Supplementary Fig. S6J). A significant antiproliferative effect was observed in both medulloblastoma cells (Fig. 3J and Supplementary Fig. S6K) stably expressing cMET shRNA compared with nonsilencing control cells. Furthermore, a significant inhibition of HGF-mediated invasion was observed (Fig. 3K and Supplementary Fig. S6L). Interestingly, the relative inhibition of invasion in cMET shRNA expressing Daoy cells mimics the treatment of Daoy cells with 500 nmol/L to 1 μmol/L of foretinib (Fig. 3H).

Collectively, these results suggest that foretinib exhibits a significant in vitro inhibitory activity in medulloblastoma cells, mainly through blockade of the cMET pathway.

**Foretinib induces medulloblastoma regression in vivo**

We sought to investigate the antitumor activity of foretinib in vivo using subcutaneous xenograft models of SHH medulloblastoma. Daoy and ONS76 cells were implanted in the flank of nude mice, and animals with established tumors were treated by oral gavage with vehicle (DMSO) or foretinib (60 and 100 mg/kg), once every other day for 9 days. Therapy with foretinib significantly induced tumor regression at all drug concentrations administered. At the end of treatment, tumor volumes were reduced by 37% and 46% with 60 and 100 mg/kg of foretinib, respectively, in Daoy xenografts (Fig. 4A and B) and by 50% in ONS76 xenografts (Supplementary Fig. S7A and S7B). Foretinib was well tolerated as mice body weights were maintained during the treatment period for all cohorts (Fig. 4C and Supplementary Fig. S7C). Medulloblastoma tumor growth inhibition in mice treated with foretinib was associated with concomitant reduction in cMET and PDGFRβ pathway activity (Fig. 4D and Supplementary Fig. S7D). Foretinib treatment resulted in a potent inhibition of cMET phosphorylation in all cohorts (Fig. 4E and Supplementary Fig. S7E) and a moderate reduction in PDGFRβ activation (Fig. 4F). Tumors treated with foretinib also displayed significant dose-dependent decrease in cell proliferation (Fig. 4G and Supplementary Fig. S7F), and increase in apoptosis (Fig. 4H and Supplementary Fig. S7G).

These studies demonstrate that foretinib has a significant in vivo antitumor effect in medulloblastoma allografts, through a potent suppression of the cMET pathway activity and a moderate inhibition of PDGFRβ signaling.

**Characterization of foretinib pharmacokinetics and brain permeability**

To further evaluate the potential clinical relevance of foretinib in brain tumor treatment, we assessed the pharmacokinetics of the drug and its ability to penetrate into the brain. Nude mice were treated by oral gavage with 30, 60, and 100 mg/kg of foretinib, and blood and the perfused brains were harvested at specific time points. Foretinib was detected in samples from treated animals using high-performance LC/MS/MS. Maximum concentrations of foretinib in the plasma and in the brain of mice occurred 5 hours after oral administration with no difference between dose levels (Fig. 5A and B). The same pharmacokinetic features were previously reported in a phase 1 clinical trial of foretinib in adult patients with advanced solid tumors outside the central nervous system (17). Furthermore, after 5 consecutive days of treatment with 60 mg/kg of oral foretinib, the penetration of the drug in the brain was approximately 14% (Supplementary Table S1).

We then assessed the distribution of foretinib in different mouse organs using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometer (MALDI-TOF) imaging. Foretinib produces a strong signal (Fig. 5C and D) and five hours after oral administration, when in each brain the maximum concentration, the drug is seen in the liver and also in the renal cortex (Fig. 5E). The concentration of foretinib in the brain after oral gavage was below the threshold detection of MALDI-TOF imaging.

To explore the potential use of foretinib for intrathecal therapy in neuro-oncology, we used osmotic pumps to deliver the drug into the lateral ventricles of mice. After injection of 6 mg/kg of foretinib for 28 days at a rate of 0.25 μL/hour, the drug was distributed through the cerebrospinal fluid to the supra- and infratentorial compartments (Fig. 5F; ref. 37). Foretinib was detected by MALDI-TOF imaging in the right frontal lobe of mice due to its very high concentration at the drug delivery site (Fig. 5G). The drug was well tolerated and the mice did not display any signs of central nervous system toxicity.

Collectively, these studies demonstrate for the first time, to our knowledge, that foretinib crosses the blood–brain barrier and can be safely administered as intrathecal therapy.

**Foretinib reduces SHH medulloblastoma growth and dissemination in mouse xenografts**

Having established that foretinib can effectively be delivered in the brain, we investigated its efficacy in mouse models of disseminated medulloblastoma. Daoy cells expressing luciferase were orthotopically implanted into the fourth ventricle of nude mice. Animals with a detectable signal by bioluminescence at 3 days after inoculation were treated by oral gavage either with vehicle (DMSO) or 60 mg/kg of foretinib once daily, 6 days a week, for 2 weeks. The weekly evaluation of tumor growth and dissemination by bioluminescence showed a significant...
Figure 3. cMET targeting by foretinib in vitro. A and B, foretinib inhibits cMET pathway activation and downstream signaling as determined by Western blot and chemiluminescence densitometric analysis. Densitometric analysis represents the phosphorylated proteins compared with total proteins. C, proliferation of Daoy cells treated with increasing doses of foretinib for 4 days. D and E, representative Western blot and cleaved caspase 3/7 assays demonstrating induction of apoptosis after foretinib treatment in Daoy cells. RFU, relative fluorescence units. F, representative images of a radial migration assay (magnification, ×2.5). G and H, foretinib inhibits HGF-dependent cell migration (G) and invasion (H) in Daoy cells. (Continued on the following page.)
Figure 4. Foretinib induces in vivo regression of medulloblastoma. A and B, nude mice with Daoy hind flank xenografts were treated with vehicle control (10% DMSO, n = 6) or foretinib (60 and 100 mg/kg; n = 6 per group) once every other day for 9 days. Effect of foretinib on tumor growth was compared during treatment (A) and in the end of treatment (B). Data represent group means (\(\pm\) SEM (\(\ast\), \(P < 0.001\)). C, average animal body weight before and after therapy. Data represent group mean (\(\pm\) SEM (\(\ast\), \(P < 0.001\)). D, flank tumors treated with foretinib show inhibition of p-cMET and p-PDGFR\(_{\beta}\), decreased cell proliferation (Ki-67 staining), and increased apoptosis (cleaved caspase 3 staining). Scale bar, 100 \(\mu\)m. E-H, immunohistochemical quantification of p-cMET (E), p-PDGFR\(_{\beta}\) (F), Ki-67 (G), and cleaved caspase 3 (H). Data represent mean (\(\pm\) SEM (\(\ast\), \(P < 0.05\); \(\ast\ast\), \(P < 0.01\); \(\ast\ast\ast\), \(P < 0.001\)).

Reduction in tumor size and metastases in the animals treated with foretinib (Fig. 6A and B), as confirmed by the smaller increase in the total photon flux from luciferase expressing Daoy xenografts (Fig. 6C). Histologic examination [hematoxylin and eosin (H&E) stain] of brains and spinal cords of animals in the control group showed a higher number of metastases in the ventricles and leptomeningeal spaces, as well as surrounding the spinal cord and the nerve roots, when compared with foretinib-treated animals (Fig. 6D and E).

These results demonstrate that foretinib is effective in reducing tumor growth and treating established medulloblastoma metastases in orthotopic mouse models of disseminated medulloblastoma.

Foretinib is effective against the primary and the metastatic compartments in a transgenic model of metastatic SHH medulloblastoma

We then tested the efficacy of foretinib in a recently published metastatic mouse model of SHH medulloblastoma (2). The authors used the SB transposon system where random insertion events in the cerebellar progenitor cells of Ptch\(^{+/-}\) mice induce a very aggressive form of medulloblastoma with high incidence of leptomeningeal dissemination by 10 weeks of age (2). SB medulloblastomas express high levels of cMET, HGF, and other cMET pathway activators (Fig. 7A and B). We asked whether early and continuous treatment with foretinib could prevent the formation of metastases and improve survival in this mouse model. Osmotic
pumps loaded with 6 mg/kg of foretinib or vehicle (10% cremophor) were implanted in 4 to 5 weeks old mice and delivered the drug for 28 days, at a rate of 0.25 μL/hour. Strikingly, foretinib-treated animals showed a significant increase in survival (Fig. 7C) and displayed medulloblastomas in the cerebellum with a less invasive phenotype when compared with controls (Fig. 7D and E). Moreover, treatment with foretinib reduced the incidence of metastases by 36% (Fig. 7F) and effectively blocked cMET activation (Fig. 7G and H). Mice tolerated intrathecal administration of foretinib, although 4 animals presented with intratumoral hemorrhage in the cerebellum (2 animals in the vehicle control group and 2 animals in the foretinib-treated group).

Taken together, these results demonstrate that foretinib is effective both against the primary and the metastatic compartments in medulloblastoma, by reducing primary tumor invasion and preventing leptomeningeal dissemination in an aggressive mouse model of metastatic SHH medulloblastoma.

Discussion

We show here that activation of cMET signaling is a hallmark feature of SHH medulloblastomas and correlates with poor outcome in pediatric patients. Targeting the cMET receptor with foretinib, a kinase inhibitor that crosses the blood–brain barrier, significantly reduces primary medulloblastoma growth and invasion, diminishes the incidence of metastases, and increases survival in disseminated mouse models of SHH medulloblastoma. Our study identifies for the first time a subgroup-specific targeted drug with promising efficacy against both medulloblastoma primary tumors and metastases.

We demonstrate that cMET is highly expressed in SHH medulloblastomas although the mechanisms leading to cMET up-regulation in this subgroup remain unclear. Of 332 medulloblastomas recently sequenced, there were no recurrent mutations or amplifications of the cMET gene (32, 38–40). These results suggest that the high expression of cMET is not driven by CNAs but may relate to the specific signaling events or cell of origin of the SHH subgroup. Interestingly, our previous finding that SPINT2, an inhibitor of cMET, is silenced by promoter methylation (10) and the recent publication that cMET is among the most significantly hypermethylated genes in group 4 tumors (41) suggest that cMET regulation in medulloblastomas may be driven by epigenetic factors.
Examination of molecular pathways characterizing SHH tumors with high and low cMET expression revealed distinct patterns of alteration. Although low cMET SHH medulloblastomas were associated with deregulation of biologic processes involved in neurogenesis and neurotransmission, high cMET SHH medulloblastomas were characterized by alterations in a number of cancer-related networks, namely cell migration, cell cycle, DNA repair, and transcription. The activation of distinct biologic pathways in high cMET SHH medulloblastomas supports the existence of a different cell of origin for these tumors, in keeping with a recent study that has shown a specific population of Nestin-expressing neuronal progenitors in the cerebellum can give rise to SHH-driven medulloblastomas (42).

We identify a gene signature of cMET activators, including cMET and GAB1, unique to SHH-driven tumors. Our results are supported by a previous publication where immunoreactivity for GAB1 was reported to be a surrogate marker for SHH medulloblastomas (43). Furthermore, we demonstrate that the cMET pathway activation status can segregate patients with SHH medulloblastomas into distinct prognostic outcomes. In pediatric patients with SHH tumors, activation of cMET is correlated with an increased rate of relapse and a shorter progression-free survival. Notably, this association is not seen in adult SHH patients confirming the previously reported clinical and molecular distinction between adult and pediatric SHH medulloblastomas (44). Furthermore, p-cMET status does not correlate with the presence of metastases or TP53 mutations, two known markers of

Figure 6. Foretinib decreases tumor growth and metastases in medulloblastoma xenografts. A and B, representative bioluminescence imaging of nude mice intraventricular xenografts treated with vehicle control (10% DMSO, n = 11; A) or foretinib (60 mg/kg, n = 11; B) orally once daily for 2 weeks. C, foretinib decreases medulloblastoma growth as denoted by a smaller change in total photon flux. Data represent group mean ± SEM. D, foretinib-treated intraventricular xenografts show significant reduction in the total number of metastases. Data represent group mean ± SEM (**, P < 0.01). E, representative H&E analysis of brain and spinal cord samples in the control group and the foretinib-treated group. Arrows denote metastatic deposits in a representative animal from the control group, whereas arrowheads denote metastases, smaller in number and size of a representative animal from the foretinib-treated group. Scale bar, 1,000 μm.
Figure 7.
Foretinib prevents metastases formation and increases survival in a transgenic mouse model of metastatic SHH medulloblastoma. A, heatmap illustrating aberrant overexpression of cMET activators in medulloblastomas from Ptch<sup>+</sup>/SB11/T2Onc mice with SB transposition (Ptch<sup>+</sup>/SB11/T2Onc). B, cMET and HGF are highly expressed in medulloblastomas from Ptch<sup>+</sup>/SB11/T2Onc mice when compared with normal cerebellum (CB). C, Kaplan–Meier survival curves demonstrate that Ptch<sup>+</sup>/SB11/T2Onc mice treated with foretinib by osmotic pump infusion have an increased survival. The gray bar indicates the duration of treatment. D and E, primary medulloblastomas treated with foretinib (D) display a less invasive phenotype as denoted by the dashed line separating the tumor (T) and the normal (N) cerebellum in H&E sections, and by the quantification (E) of the percent tumor invasion. F, foretinib decreases the incidence of metastases in an aggressive model of metastatic medulloblastoma. Data represent group mean ± SEM. G and H, foretinib pump infusion inhibits cMET activation in mice bearing SB medulloblastomas. Scale bar, 5 μm.
poor prognosis across medulloblastoma subgroups (45) and within the SHH subgroup (46), respectively. We show that a simple immunohistochemical analysis of activated cMET provides a high-quality clinical trial biomarker for identification of patients who could benefit from targeted therapy using cMET inhibitors.

Foretinib had dramatic therapeutic effect in SHH medulloblastoma, both in vitro and in vivo. Furthermore, our pharmacokinetic studies demonstrate for the first time that foretinib penetrates the blood–brain barrier and is well tolerated and distributed through intrathecal administration. Foretinib reduces primary medulloblastoma growth and invasion, and has also a potent activity against medulloblastoma metastases. Administration of foretinib to an aggressive metastatic mouse model of SHH medulloblastoma increases survival by 45% and diminishes the incidence of metastases by 36%. These tumors express high levels of HGF, suggesting an autocrine signaling loop that maintains cMET pathway activation. Previous studies on glioblastoma have shown a correlation between HGF autocrine expression by tumor cells and an increased sensitivity to cMET inhibition, which may explain the efficacy of foretinib (47). Interestingly, we show that foretinib also decreases activation of AKT, a downstream effector of both cMET and PDGFRβ signaling, and recently identified as a key contributor to leptomeningeal dissemination in medulloblastoma (2, 3). Therefore, the antitumor properties of foretinib may be related to its unique ability to target three key drivers in medulloblastoma dissemination, namely cMET, PDGFRβ and indirectly, PI3K.

Given the high expression of cMET in SHH-driven medulloblastomas, the therapeutic regimen that may offer maximum benefit in this subset of patients is the combination of foretinib with an antagonist of SHH signaling. A significant antitumor effect was reported in a patient with metastatic medulloblastoma after inhibition of smoothened (SMO), a critical component of SHH pathway, although the response was transient due to the emergence of acquired resistance (48). Thus, we anticipate that combining foretinib with a SMO inhibitor may have a synergistic and an anti-resistance effect treating cMET-dependent SHH medulloblastomas.

In summary, we show that the drug foretinib is effective treatment in preclinical murine models of metastatic SHH medulloblastoma. Given the dismal outcome and lack of options for these patients, our results provide strong rationale for repurposing foretinib as a targeted agent in SHH-driven medulloblastoma.

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

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