Discovery of AMG 458: A Potent, Selective, and Orally Bioavailable c-Met Inhibitor

Deborah Choquette, Longbin Liu, Tae-Seong Kim, Mark Norman, Aaron Siegmund, Ning Xi, Steven F. Bellon, April Chen, Paula Kaplan-Lefko, Matthew Lee, Jasmine Lin, Karen Rex, Yohannes Teferra, Isabelle Dussault, Jean-Christophe Harmange

Abstract

A novel series of pyrazolones was recently identified as potent inhibitors of c-Met. Increased selectivity over VEGFR-2 was achieved with the incorporation of a 2-hydroxypropyl group in the N1 position of the pyrazolone ring. Analysis of both in vitro and in vivo studies revealed oxidation of the hydroxyl group to form the corresponding ketone; an active metabolite lacking selectivity over VEGFR-2. Subsequent modification of the 2-hydroxypropyl group led to the discovery of 1-(2-Hydroxy-2-methylpropyl)-N-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazole-4-carboxamide (AMG 458), a molecule that exhibits a favorable pharmacokinetic profile in mouse, rat, dog, and monkey. Moreover, AMG 458 significantly inhibited tumor growth when dosed orally in the TPR-Met and U-87 MG xenograft models with no adverse effect on body weight. As a result, AMG 458 was progressed as a potential clinical candidate for the treatment of human cancers.

Background

c-Met is a receptor tyrosine kinase that is activated by its ligand, hepatocyte growth factor/scatter factor (HGF/SF) (1, 2). Upon activation, the intracellular C-terminal docking domain recruits and subsequently activates a wide range of downstream signaling molecules that contribute to the survival, proliferation, migration, and invasion of cells (3-5). Transient activation of c-Met by HGF through a paracrine mechanism is important during embryogenesis and tissue repair. However, over-expression of HGF and/or c-Met or activating mutations of c-Met have been linked to human cancers. Among various approaches that target the c-Met/HGF signaling pathway (6-9), inhibition of the c-Met kinase activity with small molecules has the potential to block both the ligand-dependent and ligand-independent activation of c-Met. This approach has recently been supported by the reported anti-tumor activity of a small molecule inhibitor of c-Met in a variety of c-Met driven human tumor xenograft models (10).
Clinical and Translational Leads

We were intrigued by the structural features of acylthiourea A (Fig. 1) when disclosed in the patent literature (11) as a c-Met kinase inhibitor. Structural similarities with kinase inhibitors such as imatinib were observed indicating the potential for binding to the inactive form of the kinase (12). In an effort to reduce potential liabilities associated with the acylthiourea functionality and conformationally restrain the molecule while maintaining key hydrogen bonds with the protein; the acylthiourea was replaced with an aminoacylpyrazolone ring.

The initial N1-methyl pyrazolone was identified as a potent c-Met inhibitor, but demonstrated minimal selectivity against VEGFR-2 (Table 1, compound 1). The replacement of the N1 methyl group with a propyl group (compound 2) significantly enhanced selectivity; however, further branching to an isopropyl moiety (compound 3) resulted in reduced activity at the cellular level. Interestingly, the introduction of a secondary hydroxyl group (compounds 4 and 5) maintained both enzyme and cellular potency relative to compound 1, independent of stereochemistry.

Having identified potent and selective inhibitors of c-Met, we examined the in vivo pharmacokinetic profiles of compounds 4 and 5. When dosed iv in rats, enantiomers 4 and 5 exhibited low clearance (CL < 200 mL/h/kg; t_{1/2} 1.9–2.6 h) and good oral bioavailability (F > 50%). To assess the pharmacological activity of pyrazolone 4, inhibition of HGF-mediated c-Met phosphorylation in mouse liver was measured after oral administration. At 30 mg/kg, compound 4 inhibited 98% of c-Met phosphorylation in mouse liver at 6 hours post-dose. Detailed analysis of plasma samples revealed the presence of significant amounts of ketone 6. When dosed orally in mice, compound 4 was quickly converted to the corresponding ketone 6 resulting in a comparable overall exposure for both the alcohol and the ketone. These findings raised concerns that as a potent c-Met inhibitor (c-Met IC\textsubscript{50} 2 nM; IC\textsubscript{50} (PC3) 48 nM) with no selectivity over VEGFR-2 (VEGFR-2 IC\textsubscript{50} 40 nM; IC\textsubscript{50} (HUVEC) 86 nM), ketone 6 would likely contribute significantly to pharmacological activity.

To eliminate the formation of ketone 6 and thereby provide a more metabolically stable compound, the corresponding tertiary alcohol was prepared. Tertiary alcohol 7 maintained
AMG 458: A Selective, Orally Bioavailable c-Met Inhibitor

In an attempt to improve the overall potency and properties of compound 7, the SAR of this series was further expanded and led to the orally bioavailable and selective c-Met inhibitor AMG 458 (8). Compound 8 potently inhibited c-Met at both the enzymatic and the cellular levels (c-Met IC$_{50}$ 2 nM; IC$_{50}$ (PC3) = 60 nM; Table 1) with no loss of selectivity over VEGFR-2. In addition, compound 8 demonstrated 100-fold selectivity when tested against a panel of 55 tyrosine and serine/threonine kinases. However, compound 8 significantly inhibited Ron, a tyrosine kinase closely related to c-Met, as well as five of the c-Met activating mutations found in human cancers (13, 14).

### Table 1. Potency and selectivity of N1-substituted pyrazolones.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>A</th>
<th>IC$_{50}$ (nM)</th>
<th>c-Met</th>
<th>PC3*</th>
<th>VEGFR-2</th>
<th>HUVEC†</th>
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*IC$_{50}$ value for HGF-mediated c-Met phosphorylation in PC3 cells (n > 4)

†IC$_{50}$ value for VEGFR-2-mediated survival of human umbilical vein endothelial cells (n > 2).
Due to the excellent in vitro potency and selectivity of compound 8, we assessed the in vitro metabolic stability across species. Compound 8 displayed good metabolic stability in mouse, rat, dog, monkey and human liver microsomes with low intrinsic clearance (<5 and 62, 8, 8, 18 µL/min/mg, respectively). In vivo, inhibitor 8 exhibited low clearance in mouse, dog, and monkey, and low to moderate clearance in rat (Table 2). Although compound 8 was rapidly eliminated in mouse and rat with a terminal half life less than 2 hours, a more sustained exposure was achieved in dog and monkey with half lives ranging from 3 to 6 hours, respectively. When administered orally, compound 8 achieved remarkably high bioavailability in all species.

Given the good pharmacokinetic profile of compound 8, the inhibition of c-Met phosphorylation in vivo was evaluated in a mouse liver pharmacodynamic assay. Compound 8 inhibited HGF-mediated c-Met phosphorylation in a dose-dependent manner with an approximate ED$_{90}$ of 30 mg/kg and an associated exposure of approximately 15 µM at 6 hours (Fig. 2A). Good correlation was observed between the inhibition of c-Met phosphorylation in liver and plasma concentration.

The anti-tumor effect of compound 8 as a single agent was then examined in two xenograft models. Inhibitor 8 was first evaluated in a tumor model derived from NIH-3T3 cells transfected with TPR-Met, a constitutively active ligand-independent form of c-Met (15). In the NIH-3T3/TPR-Met model, compound 8 exhibited dose-dependent tumor growth inhibition when administered orally with an ED$_{50}$ of -12 mg/kg (95% CI = 9-16) with an associated AUC$_{(0-24)}$ of 96 µM*h and an ED$_{90}$ of -34 mg/kg (95% CI = 12-94) with an associated AUC$_{(0-24)}$ of 241 µM*h (Fig. 2B). Compound 8 was further evaluated in the U-87 MG glioblastoma xenograft tumor model in which c-Met activation is induced via binding of its ligand, HGF (autocrine loop) (16, 17). In this ligand-dependent model, compound 8 inhibited tumor growth when dosed orally with an ED$_{50}$ of -16 mg/kg (95% CI = 9-27) with an associated AUC$_{(0-24)}$ of 130 µM*h and an ED$_{90}$ of -59 mg/kg (95% CI = 22–160) with an associated AUC$_{(0-24)}$ of 482 µM*h (Fig. 2C). In both xenograft models, tumor growth inhibition was achieved without body weight loss. These encouraging results combined with favorable pharmacokinetic profiles across species warranted advancement of AMG 458 (Table 1; compound 8) as a potential clinical candidate.

### Table 2. Pre-clinical pharmacokinetic properties of AMG 458 across species.*

<table>
<thead>
<tr>
<th></th>
<th>Mouse (Balb/c)</th>
<th>Rat (Sprague Dawley)</th>
<th>Dog (Beagle)</th>
<th>Monkey (Cynomolgus)</th>
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<tr>
<td>CL (L/h/kg)</td>
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<td>0.73</td>
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<td>V$_{ss}$ (L/kg)</td>
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<td>0.62</td>
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<td>t$_{1/2}$ (h)</td>
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<tr>
<td>F (%)</td>
<td>100</td>
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*i.v. dose: 1 mg/kg (20% captisol with pH adjusted to 3.5 using methanesulfonic acid), p.o. dose: 10 mg/kg (2% HPMC and 1% Tween 80 with pH adjusted to 2.2 using HCl). Both were solution formulations with the same drug concentration of 1 mg/mL.*
Fig. 2. PD and efficacy studies of AMG 458 (Table 1; compound B) administered orally. (A) Inhibition of HGF-mediated c-Met phosphorylation at 6 h in mouse (Balb/c) liver. Terminal plasma concentration of AMG 458 is indicated by the black circles. (B) Tumor growth inhibition in the NIH-3T3/TPR-Met xenograft model in nude mice. The AUC$_{(0-24)}$ at ED$_{50}$ and ED$_{90}$ were 96 and 241 µM*h, respectively. (C) Tumor growth inhibition in the U-87 MG xenograft model in nude mice. The AUC$_{(0-24)}$ at ED$_{50}$ and ED$_{90}$ were 130 and 482 µM*h, respectively. Arrow denotes the first day of dosing.
References

[A recent report on the pharmaceutical industry’s effort in developing cancer therapy targeting the c-Met pathway.]
[Includes a diagram of locations of c-Met mutations in human cancers.]
[The only other enzyme significantly inhibited by AMG 458 is the c-Met related protein RON (IC50 = 8nM). RON has also been implicated in human cancers.]