The Orally Bioavailable c-Met Inhibitor PF-2341066 Inhibits Osteosarcoma Growth and Osteolysis/osteoid Production in a Xenograft Model

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Introduction
Osteosarcoma (OS) is the most common primary bone tumor in children and adolescents. Ninety percent of patients who present with metastatic and thirty to forty percent of patients with non-metastatic disease will experience relapse, creating an urgent need for novel therapeutic strategies. The c-Met receptor tyrosine kinase and its ligand hepatocyte growth factor (HGF) are important for mitosis, motility and cell survival. Up-regulation of c-Met/HGF signaling via receptor over-expression, amplification, or mutation drives the proliferation, invasiveness and metastasis of a variety of cancer cells, including OS, prompting the development of c-Met/HGF inhibitors. OS cells depend on c-Met over-expression as introduction of dominant negative c-Met (lacking the intracellular domain) inhibits the ability of these cells to form tumors in nude mice. Despite the clear importance of c-Met/HGF signaling in the development and maintenance of OS, the potential efficacy of pharmacologic c-Met inhibition in OS has only been addressed in in vitro studies. PF-2341066 is an orally bioavailable, selective ATP-competitive inhibitor of both the Met and anaplastic lymphoma kinases (ALK). In a recent phase I clinical trial evaluating PF-2341066 in non-small cell lung cancer (NSCLC) patients, high response rates were observed. We hypothesized that PF-2341066 would inhibit the malignant phenotype of human OS cells in vitro and primary OS xenograft growth in vivo.

Materials and Methods
Analysis of cell proliferation
Human TE85, MNNG, or LM7 OS cells and immortalized fetal osteoblasts (hFOB) were plated at 37,500 cells per well in 12-well plates and treated with vehicle or PF-2341066 for three days. Media and drug were changed each day until cells were harvested by trypsinization. Total cell number was measured using a Cellometer™ Auto T4 cell counter.

Matrigel invasion assay
MNNG cells were plated in 6-well Becton Dickinson BioCoat™ Matrigel™ invasion chambers at 25,000 cells per well/insert. After 22 hours, the cells and Matrigel™ in the upper portion of the invasion chamber insert were removed and cells on the bottom of the insert were fixed in methanol and stained with crystal violet.

Primary osteosarcoma xenograft assay
Animal experiments were performed with the approval of the IACUC at the University of Rochester. MNNG cells were trypsinized and resuspended in sterile Hank’s balanced salt solution (Gibco 14175-095) at a concentration of 1,000,000 cells per 5 µL and injected into the proximal tibia of three week old female Crl:NU-Foxn1nu nude mice using a 26-gauge Hamilton syringe. The mice began receiving vehicle or PF-2341066 via oral gavage one week after MNNG xenografting (Day 7). Tumor growth was measured using electronic Vernier calipers. At the end of the assay, xenografted and contralateral control hindlimbs were harvested and fixed for analysis by micro-computed tomography (µCT) using a Scanco vivaCT40 scanner.

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Results
PF-2341066 selectively inhibited the proliferation of human OS versus osteoblasts at all but the highest concentration tested (Figure 1) and inhibited OS invasiveness (Figure 2) in vitro. Daily treatment of mice with established OS xenografts via oral gavage inhibited tumor growth (Figure 3) and associated osteolysis and osteoid production (Figure 4).

Discussion
c-Met is one of the most commonly altered tyrosine kinases in human cancer and the dependence of OS cells on up-regulated c-Met/HGF signaling represents a potential therapeutic strategy. PF-2341066 is an orally available small molecule inhibitor of c-Met and ALK that has demonstrated efficacy in a phase I clinical trial for NSCLC. PF-2341066 inhibited malignant properties of human OS cells in vitro and xenograft growth and associated lytic/blastic changes in bone in vivo, supporting its potential clinical use to treat OS.

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Figure 1. PF-2341066 selectively inhibits osteosarcoma proliferation in vitro. Human OS cells (Left) or hFOB (Right) were treated with PF-2341066 (0.1 – 3.0µM) or vehicle. Media and drug were replenished each day until cells were trypsinized and counted. * denotes statistical significance by ANOVA versus cells treated with vehicle (p<0.001).

Figure 2. PF-2341066 inhibits osteosarcoma invasiveness in vitro. MNNG cells were plated in BD BioCoat™ Matrigel™ invasion chambers and treated with vehicle (Left), or 0.1µM PF-2341066 (Right) for 22 hours. Cells on the bottom of the invasion chambers were fixed, stained with crystal violet and photographed at 200X magnification.

Figure 3. PF-2341066 inhibits osteosarcoma xenograft growth in vivo. MNNG cells were injected into the proximal tibia of Crl:NU-Foxn1nu nude mice. Mice began receiving vehicle or PF-2341066 daily via oral gavage one week after MNNG xenografting (Day 7).

Figure 4. PF-2341066 inhibits OS osteolysis/osteoid production. Representative 3-D reconstructions from µCT analysis of contralateral and xenografted hindlimbs from vehicle- or PF-2341066-treated mice.