Development and Validation of Biomarker Assays to Assess Pharmacodynamic Modulation of MET

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Introduction

- MET (cMet) proto-oncogene is a receptor tyrosine kinase that is normally activated through binding to hepatocyte growth factor (HGF). HGF-MET signaling plays a critical role in regulating tumor oncogenic processes, such as mitogenesis, survival, and invasive growth, especially in the metastatic process.
- Several selective kinase inhibitors targeting MET are currently under clinical investigation. To support and guide the clinical investigations of MET inhibitors, we describe the development of pharmacodynamic (PD) assays to assess MET modulation.
- MET has multiple phosphorylation sites that can potentially be modified by the candidate compounds. We focused on developing assays to two critical domains in MET activation: 1) the tyrosine kinase catalytic (TK) domain; and 2) the multifunctional docking site (Fig. 1)
- Phosphorylation at the TK domain (Y1234 and Y1235) regulates the catalytic activity. Phosphorylation of Y1349 and Y1356 (multifunctional docking site) results in the binding of adaptor proteins, such as Gab-1, Grb2, Shc, and c-Cbl, and the subsequent activation of signal transducers, such as PI3K, PLC-y, STATs, ERK 1 and 2, and FAK.

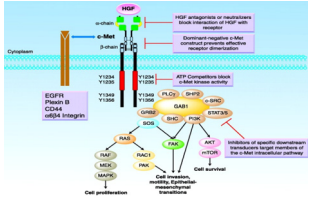

Figure 1. MET signaling and therapeutic strategies for MET inhibition. (Eder, J. P. et al. Clin Cancer Res 2009;15:2207)

Methods

MET Immunoassays. Rabbit monoclonal antibodies specific to MET, phosphorylated at Y1235 (no reactivity to Y1234) and Y1356, were generated to measure the phosphorylation status of intact MET. Intact full-length MET was purified from the membrane fraction of HEK293 cells expressing recombinant wild-type human MET (amino acids 1–1390). Antibodies against phospho-MET were combined with an N-terminal-specific capture antibody to develop ELISA (Fig. 2).

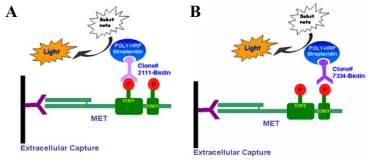

Figure 2. Immunoassay formats for the measurement of phospho-Y1235 MET (A) and phospho-Y1356 MET (B). Immunoassay for intact MET utilizes similar capture antibody and C-terminal reporter antibody (details not shown).

Tumor Pharmacodynamics. *In vivo* target modulation studies were performed in athymic nude mice bearing GTL16 (human gastric carcinoma) tumor xenografts. Mice bearing tumors of 200 mm^3 in size were given PHA665752 (NSC 748798; study 1) or vehicle (DMSO) by intraperitoneal (IP) injection, and PF02341066 (NSC 749769; Study 2) by oral gavage (PO) at the desired dose as shown in Fig. 3 (n=5–6 per group/dose/time point). Tumors were resected and processed into lysates by homogenization. All comparisons between vehicle- and drug-treated groups were performed using two-tailed unpaired students t-test (p-values ≤0.05 were considered statistically significant).

Treatment and Sampling Schedule

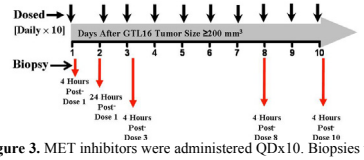

Figure 3. MET inhibitors were administered QDx10. Biopsies were collected 4 and 24 h after dose 1, and 4 h after dosing on days 3, 8, and 10.

Results

MET Immunoassay Calibration Curves

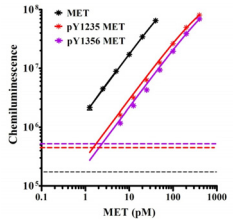

Figure 4. Calibration curves and sensitivity of MET immunoassays using intact recombinant human MET as calibrator. Hashed lines indicate the limit of quantitation (LOQ; defined as background noise +10×SD of background noise).

Analytical Performance of MET Immunoassays

	Intact MET	Phospho-Y1235 MET	Phospho-Y1356 MET
Calibration Range	1.25-40 pM	3.25-200 pM	3.25-200 pM
Inter-Assay CV (n)	<15% (5)	<15% (5)	<15% (6)
Intra-Assay CV (n)	<10% (22)	<10% (21)	<10% (12)
Dilution Recovery*	99%±12%	103%±12%	99%±11%
Spike Recovery*	98%±18%	88%±5%	86%±29%

*Data Shown as Mean±SD; CV, coefficient of variation.
Validation samples include A549, U87, and GTL16 xenograft tumor extracts

Efficacy of MET Inhibitors

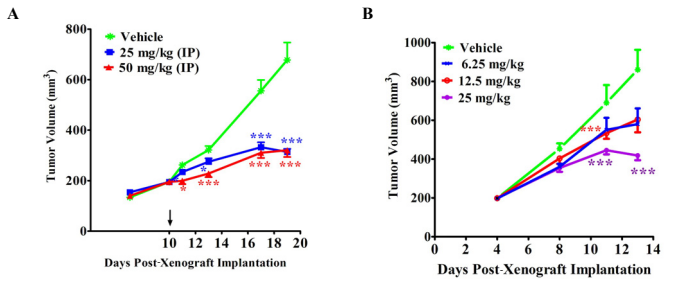

Figure 5. Mean tumor volume (± SEM) measured over time, following treatment of mice bearing GTL16 xenografts with the MET inhibitor (A) PHA665752 in Study 1 and (B) PF02341066 in Study 2. Asterisk (*) represents p-value ≤ 0.05 and ***p-value ≤ 0.01 compared to vehicle.

Intratumoral Pharmacodynamic Monitoring

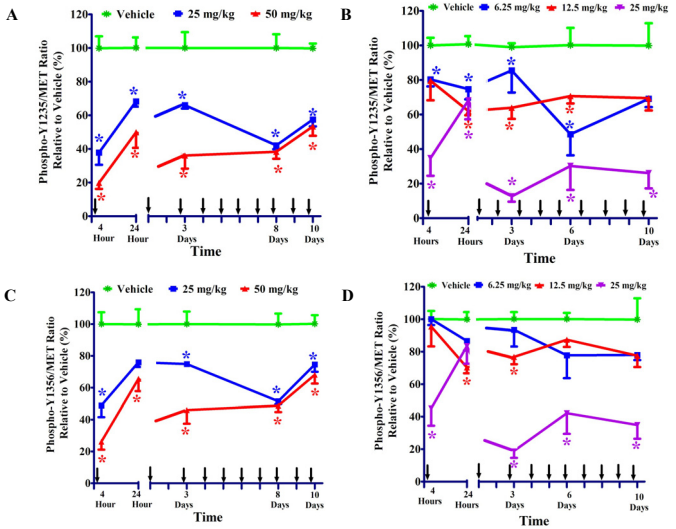

Figure 6. Mean (A–B) phospho-Y1235 MET and (C–D) phospho-Y1356 levels (± SEM) relative to vehicle (100%) in GTL16 tumor xenograft biopsies collected from 4 h to 10 days during QDx10 treatment on (A, C) Study 1 with PHA665752 or (B, D) Study 2 with PF02341066 (n = 5–6 mice/dose/time point). Asterisk (*) represents p-value ≤ 0.05 compared to vehicle. The 24-h time point indicates biomarker recovery, and arrows above the x-axis indicate dosing time. Total MET at day 10 (in the vehicle group) was 59% lower (p<0.05) for Study 1 and 36% lower (p<0.05) for Study 2 as compared to the 4-h time point.

Doses and Sampling Time Associated with Statistically Relevant Biomarker Reduction

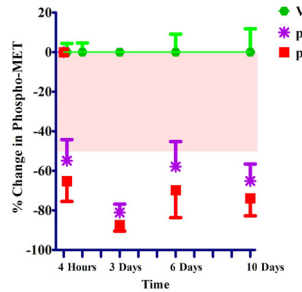

Figure 7. Phospho-MET immunoassays demonstrate statistically relevant changes (±SEM) in target at a 25 mg/kg dose of PF02341066. Surrogate for biological variation (shaded area) in phospho-MET assays were defined as follows: Biological variance = 1.96 × √2 × √(Vw)2 + (Va)2, where Vw is the average variation (CV) in vehicle-treated groups in Study 2 (PF02341066) and Va is the analytical variance (CV).

Summary and Conclusions

- We describe the development and analytical validation of novel phospho-MET PD assays. We have demonstrated the fit-for-purpose utility of phospho-MET assays to study the PD modulation of MET in tumor biopsies.
- MET PD assays are robust, accurate, sufficiently sensitive to quantify MET in tumor samples, and demonstrate target modulation using two potent MET inhibitors, PHA665752 and PF02341066, in relevant xenograft tumor models.
- Both phospho-Y1235 MET and phospho-Y1356 MET assays showed 70%–80% inhibition of phosphorylation within 4 h at most effective doses. The time course of Y1235 and Y1356 MET phosphorylation showed a sustained response. Inhibition of phospho-MET appears to correlate to the antitumor activity of MET inhibitors.
- The results of this study support the usefulness of MET assays for the PD evaluation of MET inhibitors in clinical investigations.

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All animals used in this research project were cared for and used humanely according to the following policies: the U.S. Public Health Service Policy on Humane Care and Use of Animals (2000); the Guide for the Care and Use of Laboratory Animals (1996); and the U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (1985). All NCI-Frederick animal facilities and the animal program are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.