Abstract # 11103

Application of MET Pharmacodynamic Assays to Compare Effectiveness of Five MET Inhibitors to Engage Target in Tumor Tissue

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INTRODUCTION

Several MET inhibitors that block aberrant HGF/MET signaling in different cancers are currently under clinical investigations. We utilized validated MET pharmacodynamic (PD) assays to compare time course, magnitude, and reversal of MET suppression by small-molecule MET inhibitors. We selected five MET inhibitors that were available to NCI. These agents broadly represent three major strategies for inhibiting the MET signaling pathway in cancer: 1) allosteric MET inhibitor, such as tivantinib[1] (ARQ 197); 2) kinase inhibitors selective for MET, such as EMD1214063[2]; and 3) nonselective MET kinase inhibitors, such as PF02341066, cabozantinib (XL184)[3], and GSK1363089[4] (XL880), which have broad activity against MET and other receptor tyrosine kinases. The published in vitro pharmacological profiles of these MET inhibitors are described in **Table 1**.

Table 1. MET inhibitors, their mode of action, and kinase selectivity

ID	Type of Inhibitor	IC50 MET	Other Kinases
ARQ197 (Tivantinib)	Allosteric	327 nM	RON>10 µM, PAK3=6.6 µM, Flt4=16 µM, PIM-1=33% inhibition at 10 µM
EMD1214063	ATP competitive	3 nM	Ron >10000 nM, IRAK4=615 nM, TrkA=1017 nM, Axl=1566 nM, Mer=2272 nM
Cabozantinib (XL184)	Multiple kinases ATP competitive	1.3 nM	VEGFR2=0.035 nM, KIT=4.6 nM, RET=5.2 nM, AXL=7 nM, FLT3=11.3 nM, TIE2=14.3, RON=124 nM
GSK1363089 (XL880) (Foretinib)	Multiple kinases ATP competitive	0.4 nM	VEGFR2=0.86 nM, Tie2=1.1 nM, FLT4=2.8 nM, FLT3=3.6 nM, RON=3 nM, FLT1=6.8 nM

MATERIALS AND METHODS

Preclinical Studies: Comparison of MET inhibitors was performed in the SNU5 human gastric tumor cell line (autocrine and/or autophosphorylation) model. Subcutaneous xenografts were implanted in nude mice. Once tumors reached 200–300 mm^3, the mice were given one oral dose of MET inhibitor, as shown in **Table 2**. Plasma and tumors were collected 0.5, 1, 2, 4, 6, 12, 24, 48, and 72 hours after administration of the drugs, and they were flash frozen for analysis of drug concentrations and pY$^{1234/1235}$MET inhibition.

Plasma & Tumor Pharmacokinetics: MET inhibitor levels in plasma and tumor samples were determined by Southern Research Institute (ARQ-197 and EMD 1214063) or SRI International (XL184 and XL880), using LC-MS/MS. Plasma samples were processed by organic extraction or protein precipitation; tumor fragments were minced and homogenized prior to extraction.

Analytes were separated by reverse-phase HPLC and quantified by multiple-reaction-monitoring using a triple quadrupole mass spectrometer operating in the electrospray ionization, positive-ion mode. Standard criteria for acceptable accuracy and reproducibility were applied. Limits of quantitation ranged from 10 to 100 ng/mL.

MET Assay Development & PD: MET assays (total MET, pY$^{1234/1235}$MET, and pY^{1356}MET) were developed and validated as described earlier[5]. Tumor specimens were processed for total cell lysates for MET analysis.

Statistics: All descriptive statistics (Mean, SD, CV, R^2) were calculated with Microsoft Excel and GraphPad Prism (v3.04). The significance level for the comparison between groups and the correlation between parameters was set at 95% confidence interval (CI) at $\alpha = 0.05$ for a two-sided test.

Table 2. MET inhibitor dosage regimens for PD time-course study

#	ID	NSC #	MTD (mg flat, mg/m^2, or mg/kg)	Mouse Dose 1 (mg/kg)	Mouse Dose 2 (mg/kg)	Mouse Dose 3 (mg/kg)	Mouse Dose 4 (mg/kg)
1	PF02341066 (Crizotinib)	756645	250, BID	166, QD	55, QD	28, QD	16, QD
2	ARQ197 (Tivantinib)	758242	360, BID	240, QD	80, QD	24, QD	6, QD
3	EMD1214063	758244	210 - ?	30, QD	10, QD	3, QD	1, QD
4	XL184 (Cabozantinib)	761068	175, QD	33, QD	11, QD	5.5, QD	3.3, QD
5	GSK1363089 (XL880)(Foretinib)	755775	3.6 mg/kg, QD	83, QD	28, QD	14, QD	8.3, QD

RESULTS

Pharmacokinetic (PK) Profile of Four MET Inhibitors

Table 3. Plasma and Tumor PK Parameters

Compound	Dose	$t_{1/2}$ (hr)*		AUC (hr·ng/mL)		AUC ratio
		Plasma	Tumor	Plasma	Tumor	
ARQ 197	240	6.3	15	4829	21997	4.5
EMD1214063	30	1.9	3.5	2602	89684	34.5
XL184	33	3.5	4.3	27995	66000	2.35
XL880	83	3.4	9.8	26172	171300	6.54

Tumor PK

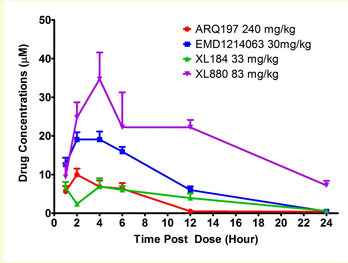

Figure 1. Intra-tumoral PK profile of four MET inhibitors. Plasma (data in Table 3) and tumor exposures were measured by LC-MS/MS in paired samples collected from mice bearing SNU5 tumors (n=3 per group). Values represent the mean of tumor tissue obtained from the maximum tolerated dose (MTD, except for EMD1214063 for which MTD is not yet published) at the indicated time points.

Tumor concentrations of MET inhibitors exceeded micromolar levels and were 2–35-fold higher than the plasma concentrations. The PK profile suggests adequate drug exposure of all MET inhibitors tested in this study.

Time Course of Changes in Intact MET in Response to MET Inhibitors

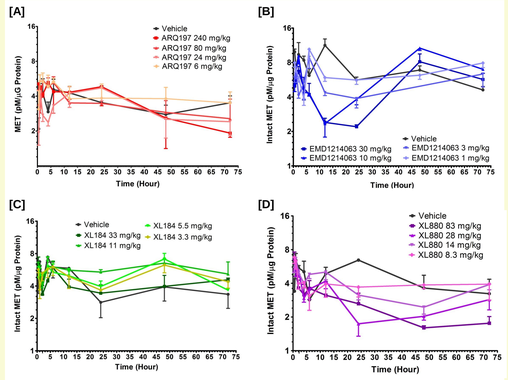

Figure 2. Changes in total MET levels after administration of a single dose. MET levels were significantly ($p<0.05$) reduced at 12h and 24h post-EMD1214063 at 10– 30 mg/kg doses, and at 24h post-XL880 at 28–83 mg/kg doses. Time on the x-axis refers to time after the single dose in the amounts indicated. The Y-axis is shown as log scale to clearly demonstrate >90% inhibition; y-axis units (pM/µg protein) refer to picomoles of MET per microgram of protein extract.

Time Course of pY$^{1234/1235}$MET Inhibition (PD Time Course)

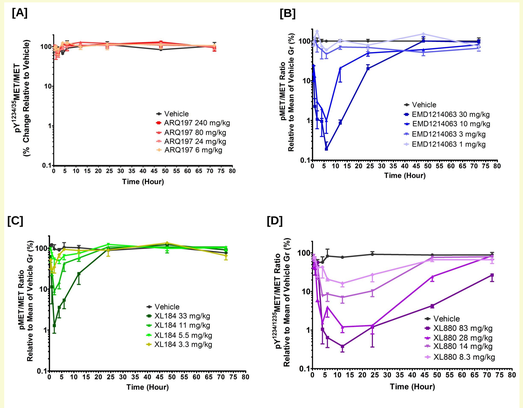

Figure 3. Time course of pY$^{1234/1235}$MET inhibition by [A] ARQ197, [B] EMD1214063, [C] XL184, and [D] XL880 at doses equivalent to MTD (except for EMD1214063), MTD/3, MTD/6, and MTD/10. The time on the x-axis refers to the time after the single administration of the indicated doses. The y-axis is shown as a log scale for clarity (differences greater than 90% inhibition). Y-axis data are shown as the ratio of pY$^{1234/1235}$MET to total intact MET relative to the mean of vehicle group.

Tumor PK Profile of MET Inhibitors and their Relationship to PD Response

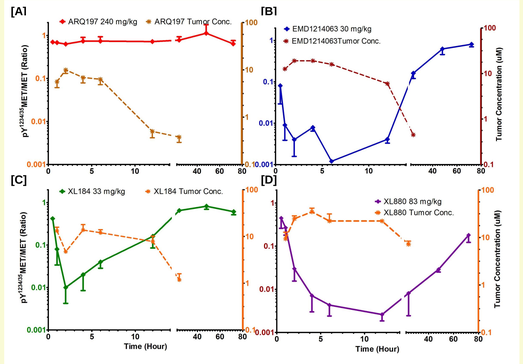

Figure 4. Relationship between mean intra-tumoral concentrations (PK) of MET inhibitors [A] ARQ 197, [B] EMD1214063, [C] XL184, and [D] XL880, and concomitant pY$^{1234/1235}$MET inhibition (PD) at the MTD. Both tumor PK and MET inhibition were analyzed in tumor quadrants collected from same xenograft. The absence of the PD response from ARQ197 was not due to lack of tumor exposure. For EMD1214063 and XL880 compounds, the PD modulation was directly related to tumor exposure. For XL184, the tumor concentrations at 4h, 6h, and 12h were higher compared to 2h time point when maximal PD response was observed. The time on the x-axis refers to time after the single dose.

Comparative PD Response of MET Inhibitors at MTD and at the Lowest Dose Producing >90% Inhibition

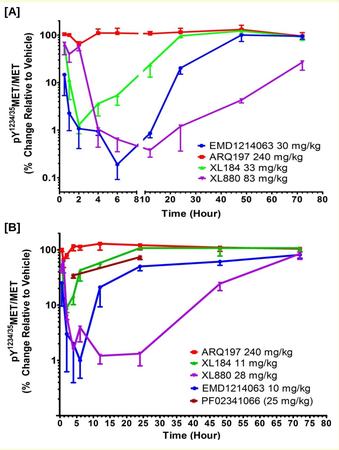

Figure 5. Comparison of time course and magnitude of pY$^{1234/1235}$MET inhibition among four MET inhibitors. Changes in levels of pY$^{1234/1235}$MET at different time points in response to MET inhibitors at (A) MTD and (B) at doses that were lower but achieved >90% inhibition (with the exception of ARQ197). Data at doses lower than MTD also include crizotinib (PF02341066), which was used as a 5th MET inhibitor for comparison.

The time on the x-axis refers to the time after the single dose in the amounts indicated in the legend.

Relationship between Tumor PK and MET Inhibition

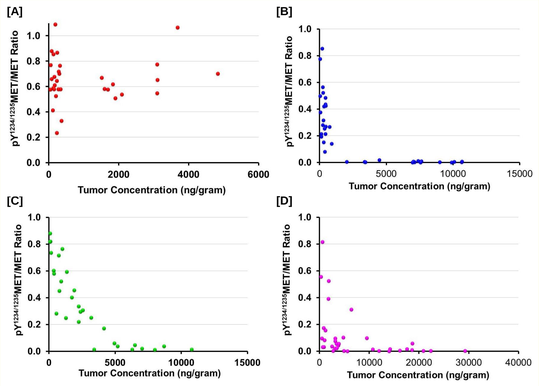

Figure 6. Relationship between intra-tumor drug concentrations and PD response of different MET inhibitors. Drug concentrations of [A] ARQ197, [B] EMD1214063, [C] XL184, and [D] XL880 in tumor tissues were measured by LC-MS/MS and related to the pY$^{1234/1235}$MET/MET ratio measured by ELISA. The PK measurements were performed in tumors from the highest and lowest dose groups (described in Table 2).

SUMMARY AND CONCLUSIONS

- We applied validated MET PD assays to directly compare similarities and differences in the extent and duration of MET kinase inhibition by five MET inhibitors in a human gastric tumor model following a single dose.
- PK monitoring revealed that tumor drug concentrations were often >10-fold higher than plasma levels. In addition, tumor exposure exceeded µM levels for all MET inhibitors.
- Time course and magnitude of pY$^{1234/1235}$MET inhibition varied considerably among MET inhibitors. Following general conclusions can be drawn:
  - Tivantinib failed to inhibit pY$^{1234/1235}$MET or intact total MET at any time point up to 72 h after drug administration.
  - EMD1214063 and foretinib appeared to inhibit intact total MET levels at 12 h and 24 h post-dose, respectively.
  - The most rapid (within 30 min) pY$^{1234/1235}$MET inhibition was observed with EMD1214063 at doses of 10 and 30 mg/kg. The MET inhibition was sustained at >90% for >12 h.
  - The most sustained (up to 48 h) pY$^{1234/1235}$MET inhibition was observed with foretinib at a dose of 83 mg/kg.
  - The multi-kinase (VEGF, RET, and MET) inhibitor cabozantinib showed biomarker recovery at 4 h even though high drug exposure was maintained up to 12 h in tumor tissue.
- Intra-tumoral PK-PD relationships indicated that no further pY$^{1234/1235}$MET inhibition was observed once tumor exposure reached certain µM levels.
- Our results provide an important foundation for a head-to-head comparison of the anti-tumor efficacies of MET inhibitors at equal MET kinase inhibition. In the next phase, we plan to determine efficacy at the lowest dosing regimen that results in 90% or greater MET inhibition. These studies could also suggest an MET pathway suppression threshold to achieve tumor regression with minimal toxicity.
- The results of this study will provide rationale for more effective application of MET inhibitors.

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