Abstract # 3691

MET Target Inhibition-Guided Efficacy in Preclinical Models

Apurva K. Srivastava[1], Melinda G. Hollingshead[2], Jeevan Govindharajulu[1], Joseph M. Covey[3], Dane Liston[3], James O. Peggins[3], Donald P. Bottaro[3,4], John J. Wright[3], Robert J. Kinders[1], Joseph E. Tomaszewski[3], James H. Doroshow[3], Ralph E. Parchment[1]

[1]Laboratory of Human Toxicology & Pharmacology, Leidos Biomedical research, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD; [2]Biological Testing Branch, National Cancer Institute, Frederick, MD; [3]Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD; and [4]Center for Cancer Research, National Cancer Institute, Bethesda, MD

Leidos Biomedical Research, Inc.

INTRODUCTION

A direct comparison of drug efficacy for the multiple agents currently in clinical development targeting MET-driven cancers would be useful for the selection of optimal treatment options. Previously, we utilized validated MET pharmacodynamic (PD) assays to compare the time course of phosphorylated-MET (pMET) suppression for five MET inhibitors (ASCO 2013). These agents included an allosteric MET inhibitor, tivantinib[1] (ARQ 197), EMD1214063[2], PF02341066, cabozantinib (XL184)[3], and GSK1363089[4] (XL880). In this study, we selected the three most potent candidates (XL880, XL184, and EMD1214063) to compare anti-tumor efficacy at a dosing schedule modeled to produce equivalent pMET (>90%) inhibition.

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 oral doses of MET inhibitors for 10 days for PD monitoring and for 21 days for efficacy monitoring. The dosing schedules were modeled on the previously described[2] PK-PD relationship, which is shown in **Figure 1**.

Single-dose PK data were used to simulate multiple-dose administrations and to select the dose/schedule predicted to maintain target tumor levels for each compound (**Figure 2**), using the simulation module at: http://home.fuse.net/clymer/graphs/pkplot.html. Since oral gavage of mice more frequently than BID was considered impractical, we used BID doses of 12.5 mg/kg for EMD1214063, 44 mg/kg for XL184, and a QDX16.5 mg/kg schedule for XL880.

Plasma and tumors were collected 4, 12, and 24 hrs after doses 1 and 14, and tissues were flash frozen for analysis of drug concentrations and total intact MET, pY$^{1234/1235}$MET, and pY^{1356}MET inhibition.

Plasma & Tumor Pharmacokinetics: 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, TTEST) 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.

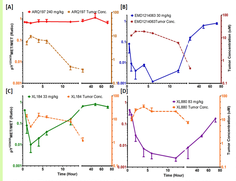

Figure 1. These data, plus additional data from lower dose groups for each drug (details not shown) were used to estimate the minimum concentrations (C_{min}) of each compound in tumors required to maintain ≥ 90% inhibition of pMET. **(Srivastava et. Al., ASCO 2013)**

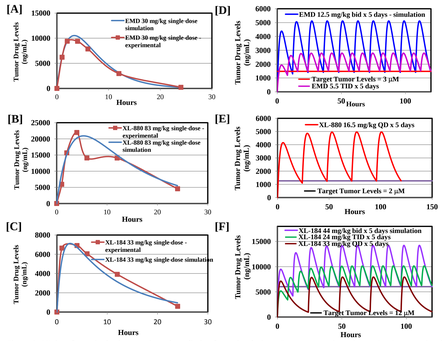

Figure 2. PK data from single-dose study and prediction from simulations. Doses were selected to maintain tumor levels above (C_{min}): XL184 = 12 μM; XL880 = 2 μM; and EMD1214063 = 3 μM.

RESULTS

Plasma and Tumor PK Profile of Three MET Inhibitors After Single & Multiple Doses

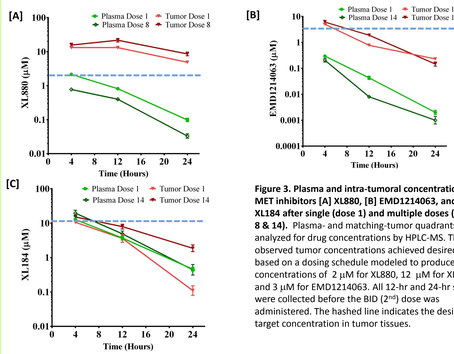

Figure 3. Plasma and intra-tumoral concentrations of MET inhibitors [A] XL880, [B] EMD1214063, and [C] XL184 after single (dose 1) and multiple doses (doses 8 & 14). Plasma- and matching-tumor quadrants were analyzed for drug concentrations by HPLC-MS. The observed tumor concentrations achieved desired levels based on a dosing schedule modeled to produce tumor concentrations of 2 μM for XL880, 12 μM for XL184, and 3 μM for EMD1214063. All 12-hr and 24-hr samples were collected before the BID (2nd) dose was administered. The hashed line indicates the desired target concentration in tumor tissues.

Changes in Intact MET Levels in Response to MET Inhibitors

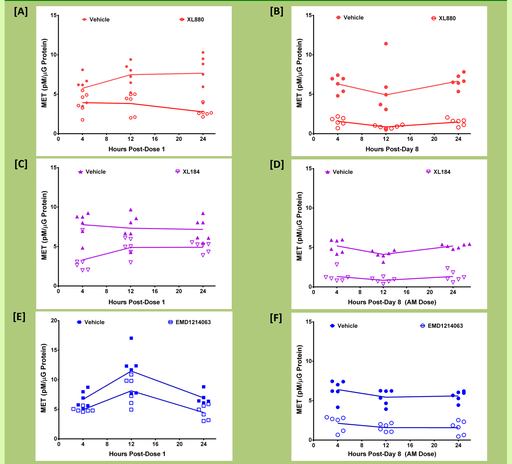

Figure 4. Changes in MET levels after single dose of [A] XL880, [C] XL184, and [E] EMD1214063, and after 8 continuous days of dosing [B] XL880, [D] XL184, and [F] EMD1214063. The time on the x-axis refers to the time after the single dose (dose 1) or dose 8 for XL880, and dose 14 for XL184 and EMD1214063. Intact MET levels were reduced by 26–64% (P<0.05 vs vehicle except XL880 4-hr and EMD1214063 12-hr time points) after the first dose and by 67–82% (p<0.01 vs vehicle) after day 8 doses. The trend line indicates the mean value of each group.

Suppression of pY$^{1234/1235}$MET by MET Inhibitors

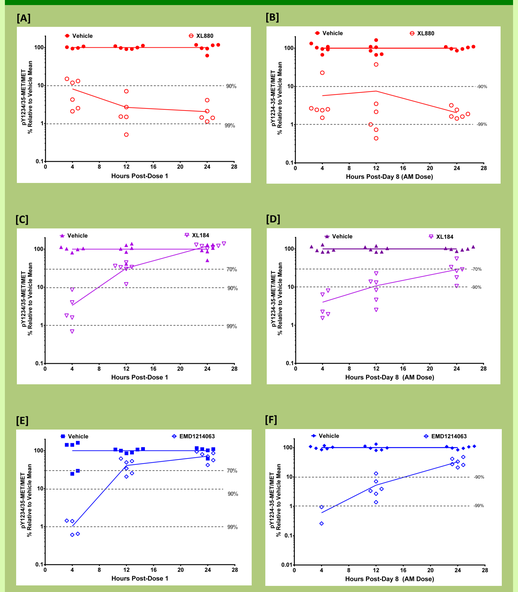

Figure 5. Inhibition of pY$^{1234/1235}$MET after single dose of [A] XL880, [C] XL184, and [E] EMD1214063, and after multiple doses of [B] XL880, [D] XL184, and [F] EMD1214063. The doses of MET inhibitors were described in the Methods section. The time on the x-axis refers to the time after dose 1 (on day 1) or dose 8 for XL880, and dose 14 for XL184 and EMD1214063 (on day 8). The pY1234/35MET/MET ratios were reduced by 92–97% (P<0.01 vs. vehicle) 4 hrs after the first dose, and by 94–99% (P<0.01 vs. vehicle for all groups) after day 8 doses. Y-axis data are shown as the ratio of pY$^{1234/1235}$MET to total intact MET relative to the mean of the vehicle group. The levels of pY$^{1234/35}$MET from several drug-treated mice fell below detection level, and account for missing data points. The y-axis is shown as a log scale for clarity (differences greater than 90% inhibition). The trend line indicates the mean value of each group.

Suppression of pY^{1356}MET by MET Inhibitors

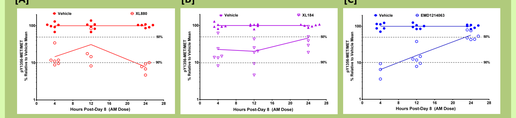

Figure 6. Inhibition of pY^{1356}MET after 8 days of continuous dosing of [A] XL880, [B] XL184, and [C] EMD1214063. The time on the x-axis refers to the time after dose 8 for XL880 and after dose 14 for XL184 and EMD1214063 (all on day 8). The pY^{1356}MET/MET ratios were reduced by 42–92% (P<0.05 vs. vehicle). Y-axis data are shown as the ratio of pY^{1356}MET to total intact MET relative to the mean of the vehicle group. The y-axis is shown as a log scale for clarity (differences greater than 90% inhibition). The trend line indicates the mean value of each group.

Tumor Growth Inhibition by MET Inhibitors

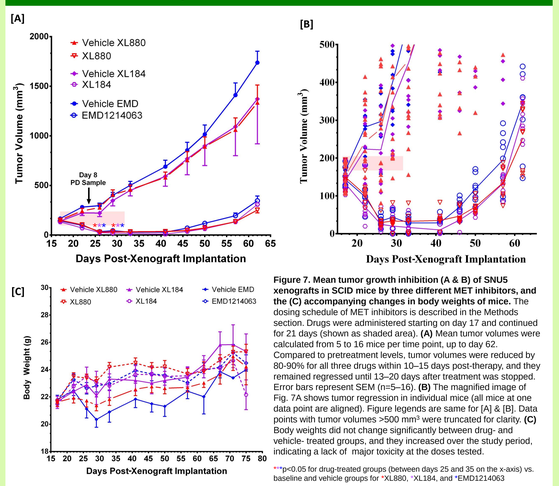

Figure 7. Mean tumor growth inhibition (A & B) of SNU5 xenografts in SCID mice by three different MET inhibitors, and the (C) accompanying changes in body weights of mice. The dosing schedule of MET inhibitors is described in the Methods section. Drugs were administered starting on day 17 and continued for 21 days (shown as shaded area). **(A)** Mean tumor volumes were calculated from 5 to 16 mice per time point, up to day 62. Compared to pretreatment levels, tumor volumes were reduced by 80-90% for all three drugs within 10–15 days post-therapy, and they remained regressed until 13–20 days after treatment was stopped. Error bars represent SEM (n=5–16). **(B)** The magnified image of Fig. 7A shows tumor regression in individual mice (all mice at one data point are aligned). Figure legends are same for [A] & [B]. Data points with tumor volumes >500 mm^3 were truncated for clarity. **(C)** Body weights did not change significantly between drug- and vehicle- treated groups, and they increased over the study period, indicating a lack of major toxicity at the doses tested.

/*p<0.05 for drug-treated groups (between days 25 and 35 on the x-axis) vs. baseline and vehicle groups for *XL880, *XL184, and *EMD1214063

Summary of Suppression of MET and Signaling Molecules Involved in MET Pathway

Table 1. Summary of MET Suppression

	Time Point (Hrs)	Reduction in Intact MET (%)	Reduction in pMET (%)	pMET as % of Total Intact MET of Vehicle Treated Group
XL880	4	75	94	1.5
	12	82	92	1.4
	24	78	98	0.4
XL184	4	75	97	0.8
	12	80	89	2.2
	24	74	71	7.5
EMD1214063	4	67	99	0.3
	12	71	95	1.5
	24	72	67	9.2

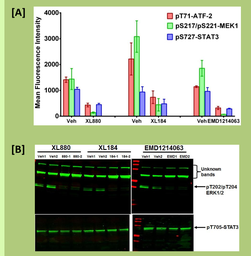

Figure 8. The effect of MET suppression by three MET inhibitors on select MET pathway signaling molecules 4 hrs post-day 8 dose. (A) The status of three downstream molecules affected by MET signaling was determined in total cell lysates from tumor quadrants (n= 2–6) by Bio-Plex Luminex assays (Bio-Rad). The 9-plex MAPK panel produced reportable results for ATF–2 (Thr^{71}), MEK1 (Ser$^{217/221}$), and STAT3 (Ser^{727}) markers. The comparison of mean MFI indicates 67–72%, 86–96%, and 49–70% decreases in signal intensity in pATF, pMEK, and pS727-STAT3, respectively. **(B)** WB analysis of total cell lysates from two different tumor quadrants to show suppression of (top panel) phospho-ERK1/2 (antibodies reactive to phosphorylated ERK1/2 Thr202/Tyr204, Cell Signaling Technology, clone#9102) and (bottom panel) phospho-STAT3 (antibodies specific for phosphorylated Tyr705-STAT3, Cell Signaling Technology, clone#9145).

SUMMARY AND CONCLUSIONS

- This study provides a head-to-head comparison of three MET inhibitors (XL880, XL184, EMD1214063) at doses and schedules modeled to produce equivalent suppression of pMET levels in tumors (PD response). A fourth MET inhibitor, ARQ197, was excluded from this study because it failed to show pMET suppression in our preceding study (Srivastava et. Al., ASCO, 2013).
- The absolute tumor levels of MET inhibitors required to achieve >90% suppression of pMET were in the range of 2–3 μM. A higher tumor exposure for XL184 was targeted because we observed a reversal in PD response at ~10 μM levels in tumor tissue.
- All three MET inhibitors showed >90% inhibition of pMET levels in tumor tissue at doses tested in this study.
- Our data showed that all three MET inhibitors showed an 80–90% reduction in tumor volumes when accompanied by >90% inhibition of pMET levels in tumor tissues.
- Our results validate two important thresholds: 1) tumor drug exposure required to achieve a desired MET suppression; and 2) MET suppression that assures tumor regression. Thus, our data also provide a direct link between a threshold MET suppression and tumor regression in cancers driven by MET.
- Our data strongly support the use of the MET PD assays to guide dosage regiment determinations. The effectiveness of PD response-guided dosing was demonstrated in achieving the desired efficacy at lower doses for XL880 and EMD1214063 than efficacious doses described previously[7-8].
- A limitation of our study is that we tested the single pMET suppression threshold (of >90%) in a model that could be sensitive to MET inhibitors. It remains to be verified if similar efficacy can be achieved at a lower pMET suppression threshold or in models where pMET expression is much lower.

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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 Frederick National Laboratory animal facilities and the animal program are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Funded by NCI Contract No HHSN261200800001, EHHSN261201100012C, and HHS261201100016C.

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