MEK162 (ARRY-162), a Novel MEK 1/2 Inhibitor, Inhibits Tumor Growth Regardless of KRas/Raf Pathway Mutations

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Abstract #

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Introduction

- Mutated, oncogenic forms of Ras and Raf are commonly found in cancer and are implicated in uncontrolled cell growth
- Tumors which harbor these mutated forms are reported to be highly sensitive to MEK inhibition
- MEK1/2, a dual-specific kinase, is downstream of both Ras and Raf and required for the activation of ERK1/2
- MEK162 is a highly selective, orally bioavailable, ATP-uncompetitive inhibitor of MEK1/2 currently under investigation in clinical trials. In previous preclinical work, MEK162 was found to be highly effective in inhibiting growth of xenograft tumors regardless of Ras/Raf pathway deregulation (Table 1)
- MEK inhibitors are reported to affect angiogenesis, through direct effects on endothelial cell proliferation, and tumor cell apoptosis, through increasing the pro-apoptotic protein BIM
- In this study, we investigate in more detail the molecular mechanisms involved in tumor cell death in the absence of Ras/Raf mutation

Table 1: MEK162 inhibits tumor growth regardless of KRas or BRaf mutation

Tumor Line	Tumor type	Mutation status		Dose/Schedule	%TGI*	Best Response†
		KRas	BRaf			
CRC25B2	colorectal	**mut**	wt	100 mg/kg, QD	<50%	-
MIA PaCa	pancreatic	**mut**	wt	30 mg/kg QD	44%	-
CRC25B3	colorectal	**mut**	wt	100 mg/kg, QD	46%	-
LoVo	colorectal	**mut**	wt	30 mg/kg QD	49%	-
NCI-H460	non-small cell	**mut**	wt	300 mg/kg QD	53%	-
Calu-6	non-small cell	**mut**	wt	30 mg/kg QD	55%	-
CRC18B2	colorectal	**mut**	wt	100 mg/kg, QD	64%	-
A549	non-small cell	**mut**	wt	30 mg/kg QD	74%	SD
CRC25098	colorectal	wt	**mut**	100 mg/kg, QD	64%	SD
COLO 205	colorectal	wt	**mut**	300 mg/kg (BIDx3)x3w	98%	CR
BxPC-3	pancreatic	wt	wt	30 mg/kg QD	66%	PR
CRC13B2	colorectal	wt	wt	100 mg/kg BID	72%	SD
NCI-H1975	non-small cell	wt	wt	100 mg/kg BID	90%	PR
HT1080	fibrosarcoma	wt	wt	100 mg/kg QD	100%	CR

Tumor cells (BxPC-3, MIA PaCa, LoVo, COLO 205, NCI-H460, Calu-6, A549, NCI-H1975 T790M mutant or HT1080) were implanted on the right flank as a cell suspension ($5x10^6$ in saline). Primary human explant tumor tissue (CRC25B2, CRC25B3, CRC18B2, CRC25098, CRC13B2 colorectal carcinoma) were continuously propagated in mice and implanted as a 1:1 suspension of minced tumor and matrigel. All explant studies were run between tumor passage 2-4 from receipt of original biopsy. All studies were performed in female nu/nu NCr or SCID-BG mice (Harlan) as best determined by individual model growth kinetics. Studies were initiated when tumors reached a minimum of 200 mm^3. Tumor size and animal body weight were measured on the indicated days over the course of each study. MEK162 was well tolerated and daily administration did not result in drug-related body weight loss exceeding 10% or death in any study. Test compound administration and tumor growth metrics are displayed for each study as indicated.

* %TGI (tumor growth inhibition) = 100(1-Wt/Wc); Wt is the mean or median tumor volume of the treated group on day X; Wc is the mean or median tumor volume of controls on day X

† Best response of individual animals where SD, PR and CR are stable disease (<150% change in tumor size at end of test compound administration), partial response (≥50% reduction in tumor relative to initial size) and complete response (100% reduction in tumor size), respectively

All *in vivo* studies were performed in accordance with IACUC guidelines and in harmony with the Guide for Laboratory Animal Care and Use.

Tumor growth inhibition and pharmacodynamic analysis of KRas/BRaf wild-type xenografts

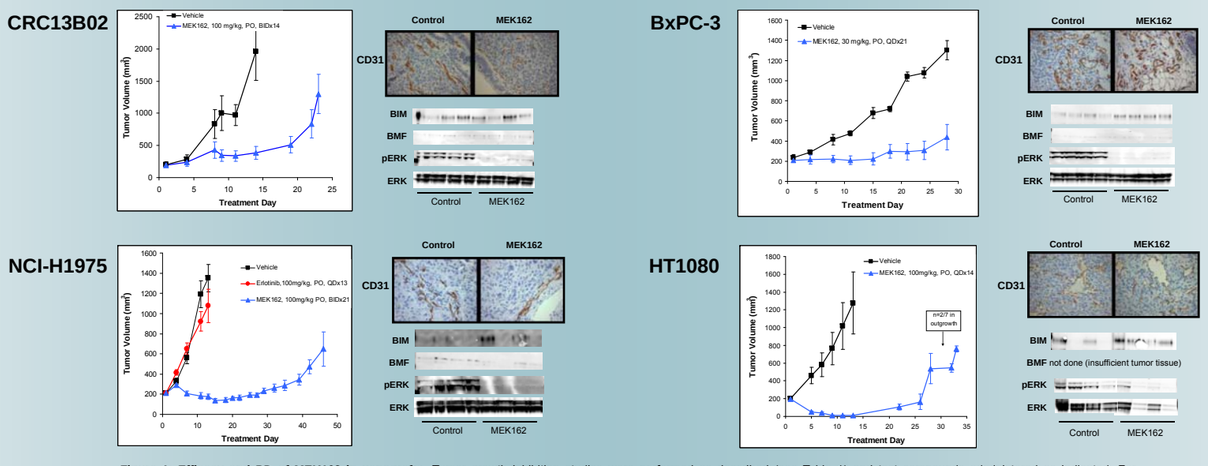

Figure 1: Efficacy and PD of MEK162 in xenografts. Tumor growth inhibition studies were performed as described (see Table 1) and test compounds administered as indicated. For pharmacodynamic analysis, tumor cells (BxPC-3, NCI-H1975 or HT1080) or tumor tissue (CRC13B02, tumor passage 2, 50% matrigel) were implanted in nu/nu NCr mice (Harlan). Mice were administered 100 mg/kg MEK162 (PO) for five consecutive days and tumors were harvested 4 hours following the final dose. Tissue was divided and placed in formalin or snap frozen in $N_2(l)$ for later analysis by immunohistochemistry or western blot, respectively. CD31 staining was performed on FFPE sections (Dianova #DIA310) and results (Table 2) are average of 5 levels counting 7 fields/level. BIM (Abcam #AB9655), BMF (Cell Signaling #2933S), pERK (Cell Signaling #9101S) and ERK (Santa Cruz #SC-94) were assessed by immunoblot.

Table 2: ERK and BIM are altered in MEK162 treated tumors while markers of angiogenesis are unaffected

	CRC13B2	NCI-H1975	BxPC-3	HT1080
In vitro efficacy results*				
in vitro EC_{50}	>10,000 nM	>10,000 nM	>5000 nM	102 nM
In vivo efficacy results				
%TGI	72%	87%	66%	100%
PR/CR/SD	2SD	1CR/2PR/5SD	1PR/4SD	7CR
In vivo pharmacodynamic results (% of vehicle-treated control on day 5)				
pERK	16%	10%	4%	49%
BIM	100%	267%	201%	134%
BMF	111%	59%	99%	n/a
In vivo angiogenesis endpoints (% of vehicle-treated control on day 5)				
CD31 staining	74%	146%	103%	97%
huVEGF†	122%	86%	124%	48%‡
msVEGF†	119%	70%	95%	120%‡

* Results of 5 day proliferation assay (n=2)
† human and mouse VEGF analyzed in tumor lysates by ELISA (human VEGF-A #BMS277/2 and mouse VEGF-A #BMS619/2, Bender MedSystems GmbH)
‡ 48 hour time point, insufficient tumor tissue remaining at day 5 time point

MEK162 causes apoptosis in HT1080 tumors through induction of BMF and BIM and suppression of BCL family members

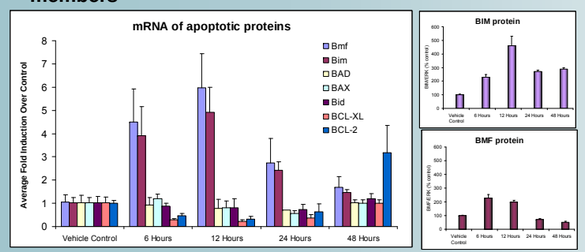

Figure 2: Analysis of apoptotic proteins in tumor xenografts following MEK162 treatment. The HT1080 tumor model exhibited the highest regression in response to MEK162 treatment and was selected for more detailed analysis of apoptotic proteins. HT1080 tumor-bearing animals were given a single dose of 100 mg/kg MEK162 and tumors harvested at the indicated time points. Tissue was snap frozen for immunoblot analysis as described above (Fig. 1) or total RNA isolation (RNeasy 96 kit, Qiagen). The RNA samples were DNase-treated and reverse-transcribed using the High-Capacity cDNA Archive Kit (Applied Biosystems, Inc.). *Real-time* PCR (7900HT Real-Time PCR System, Applied Biosystems) was performed on each sample using FAM™ or VIC™ dye-labeled Taqman® MGB probes (assay IDs: Bmf Hs00372937_m1, Bim Hs00708019_s1, BAD Hs00188930_m1, BAX Hs99999001_m1, Bid Hs00609632_m1, BCL-XL Hs00236329_m1, BCL-2 Hs00608023_m1 and GAPDH Hs99999905_m1).

MEK162 is highly effective in blocking VEGF/bFGF-induced matrigel neoangiogenesis

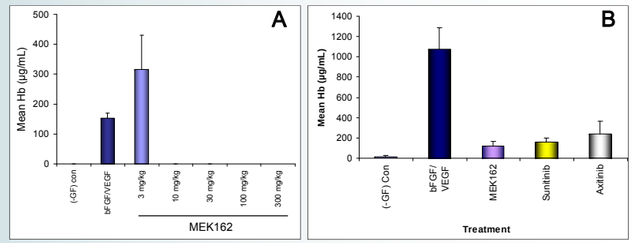

Figure 3: Inhibition of neoangiogenesis by MEK162. Female BALB/c mice (19-22 g), n=5 per treatment group, were implanted subcutaneously with 500 µL growth factor (GF) reduced matrigel (BD Biosciences, #356237) containing 500 ng each bFGF and VEGF (US Biological #F4210 and #V2110, respectively) or 0 ng as a negative control. Test compounds were administered at the doses and schedules indicated below. Animals were euthanized on day 7, and the matrigel was harvested, weighed and homogenized in phosphate buffered saline, pH 7.4. Samples were centrifuged and the resulting lysate analyzed for hemoglobin concentration by following a colorimetric reaction with tetramethylbenzidine in the presence of hydrogen peroxide and acetic acid. Data are presented as µg Hb/g matrigel, and compounds were evaluated based on the ability to inhibit GF-induced angiogenesis. A) In this model, MEK162 fully inhibited GF-induced angiogenesis at 10 mg/kg and above. B) In comparison to other angiogenesis inhibitors, MEK162 was as effective as sunitinib and axitinib which are known inhibitors of VEGFR2 and served as positive drug controls in this study.

Dosing Regimens:
Vehicle: 1% carboxymethylcellulose/0.5% tween-80, 10 mL/kg, PO, QDx7
MEK162: 3, 10, 30, 100 or 300 mg/kg, PO, QDx7
Sunitinib: 40 mg/kg, PO, QDx7
Axitinib: 25 mg/kg, IP, QDx7

Summary

- MEK162 inhibits xenograft tumor growth in the presence or absence of Ras pathway mutations
- In HT1080 tumors, MEK162 activates intrinsic cell death pathways by specific increases in BIM and BMF and decreases in BCL family members
- While MEK162 is a potent inhibitor of neoangiogenesis in the matrigel invasion assay, effects on established vascular systems are more complex. CD31 and VEGF were not affected in these models.
- Stimulation of pro-apoptotic pathways may be the major contributor to the potent anti-tumor activity observed *in vivo*.
- These data support the preclinical investigation of the effects of MEK inhibitors on apoptotic protein expression and the clinical investigation of MEK inhibitors in tumors with both wild-type and mutated Ras/Raf pathways