

# Preclinical Development of ARRY-162, A Potent and Selective MEK 1/2 Inhibitor

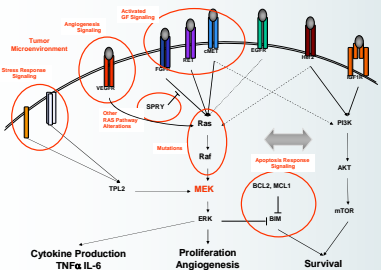
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## Introduction

- Activation of the Ras/Raf/MEK/MAP kinase pathway is implicated in uncontrolled cell proliferation and tumor growth
- Mutated, oncogenic forms of Ras are found in 50% of colon, 90% of pancreatic, and 30% of lung cancers; B-Raf mutations have been identified in more than 60% of malignant melanomas and from 40-70% of papillary thyroid cancers
- MEK, a dual specific kinase, is a key player in this pathway; it is downstream of both Ras and Raf and activates ERK1/2 through phosphorylation of key tyrosine and threonine residues.
- ARRY-162, a novel ATP-competitive inhibitor of MEK1/2, has nanomolar activity against purified MEK enzyme ( $IC_{50} = 12$  nM) and inhibits both basal and induced levels of ERK phosphorylation in numerous cancer cell lines with  $IC_{50}$ s as low as 5 nM
- ARRY-162 is especially potent at inhibiting the cell proliferation of mutant B-Raf and Ras cell lines such as HT29, Malme-3M, SK-MEL-2, COLO 205, SK-MEL-28 and A375 ( $IC_{50}$ s from 30-250 nM)
- In vivo*, ARRY-162 has demonstrated efficacy in several xenograft tumor models in mice, including HT29, BxPC3, MIA PaCa2, A549, LoVo, Calu6, DU145 and COLO 205
- ARRY-162 demonstrates good physical chemical characteristics, low clearance, medium-to high Caco-2 permeability and minimal predicted drug-drug interactions

Array's company policy is to not disclose exact structure until the compound is in Phase 2 trials. Disclosure of this structure should not impact the importance or the interpretation of the data we wish to present.

## MEK Signaling Pathway



ARRY-162 Drug Characteristics	
MW	< 450
Log $D_{50}$	2.1
<i>In Vitro</i> Hepatic CL	Low
<i>In Vivo</i> Hepatic	Low
Mouse PK %F	56 - 72
Rat PK %F	53 - 76
Monkey PK %F	30 - 59
Dog PK %F	59

## Methods

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

**Enzyme Assays**  
N-terminal 6 His-tagged, constitutively active MEK1 (2-363) was expressed in E. coli and protein is purified by conventional methods. The activity of MEK1 was assessed by measuring the incorporation of  $\gamma$ -<sup>32</sup>P-ATP onto ERK2 in the presence of MEK1.

**Cellular Assays**  
**ERK Signaling:** Malme-3M cells seeded in 96-well plates were incubated for 2hr with increasing concentrations of ARRY-162. pERK was measured by an in-cell Western assay using an antibody specific for phospho-p42/44 on ERK 1/2 (ph202y/tyr204) and an antibody to total ERK.  
**Proliferation assay:** Actively proliferating cells (HT29, Malme-3M, SK-MEL-2, COLO 205, SK-MEL-28 and A375) seeded in 96 well plates were incubated with increasing concentrations of ARRY-162 for 72 hours. Cell viability or proliferation was measured using CellTiter-Blue (Promega) percent proliferation was calculated relative to the DMSO control.

***In Vivo* Tumor Growth Studies**  
For all studies, Tumor cells were implanted in female nude mice (Taconic Laboratories, Inc. or Charles River Laboratories, Inc.) subcutaneously in the flank, and the tumors were allowed to grow to 150-200 mm<sup>3</sup> in size. Then, mice were randomized into treatment groups (n=6) to receive vehicle (1% CMC/0.5% Tween 80, 10 mL/kg) or ARRY-162 (3, 10 or 30 mg/kg). Dosing for all studies was oral, daily for 21 consecutive days.

**HT29 Human Colon Carcinoma (CRC):** Tumor cell inoculum was  $5 \times 10^6$   
For PK/PD study, separate groups of animals bearing HT29 tumors (~300 mm<sup>3</sup> in size) received single doses of ARRY-162 (3, 10 or 30 mg/kg) and groups of 30 mice were sacrificed at 2, 3, 8, 12 or 24 hr post-dose. Blood samples for ARRY-162 concentration analysis by LC/MS/MS and tumors for pERK western blotting were obtained from each animal.

**COLO205 Human Large Intestine Carcinoma (CRC):** Tumor cell inoculum was  $1.4 \times 10^7$

**BxPC3 Human Pancreatic Carcinoma:** Tumor cell inoculum was  $4 \times 10^6$

**A549 Human Non-Small Cell Lung (NSCLC) Carcinoma:** Tumor cell inoculum was  $5 \times 10^6$

**ENDPOINTS DEFINED:**  
Tumors (W<sup>3</sup>L2) were measured twice weekly for all studies  
%TGI = 100(1-L2)  
PR = 50% or greater decreases in tumor size  
CR = no measurable tumor  
SD = < 50% change in tumor size

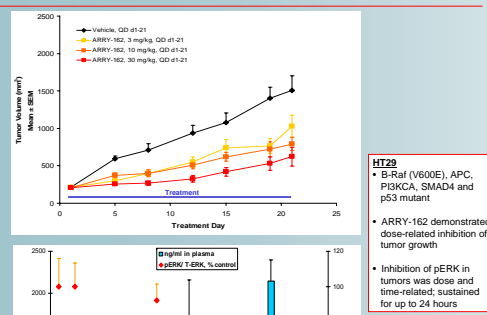
## ARRY-162: Potency and Selectivity

Potency	
MEK Enzyme $IC_{50}$	12 nM
Cellular pERK Inhibition $IC_{50}$ (Malme-3M)	11 nM
Mechanism of Inhibition	Uncompetitive with ATP
Selectivity	
Kinase Panel of 220 enzymes	No activity @ 10 $\mu$ M
Profile Screen of 30 Receptors/Ion Channels	No activity @ 10 $\mu$ M
hERG (patch clamp)	28% @ 10 $\mu$ M
P450 Inhibition (7 isoforms)	Clean - No inhibition @ 25 $\mu$ M
Genotoxicity (Ames, Micronucleus, Chrom Abt)	Negative

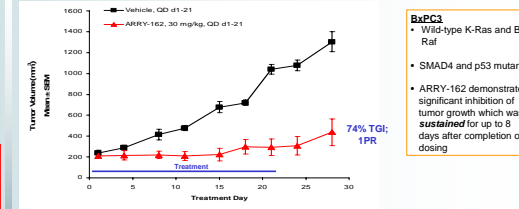
## Cellular Activity of ARRY-162

Cell Line	Tissue Type	Mutation Status	Study	$IC_{50}$ (nM)
Malme-3M	melanoma	B-Raf (V600E)	pERK	11
A375	melanoma	B-Raf (V600E)	Cell Proliferation	43
HT-29	CRC	B-Raf (V600E), APC, PI3KCA, SMAD4 and p53	Cell Proliferation	100
COLO205	CRC	B-Raf (V600E), APC, SMAD4 and p53	Cell Proliferation	44
SK-MEL-2	melanoma	N-Ras	Cell Proliferation	250
SK-MEL-28	melanoma	B-Raf (V600E), CDK and EGFR	Cell Proliferation	282
Malme-3M	melanoma	B-Raf (V600E)	Cell Proliferation	23

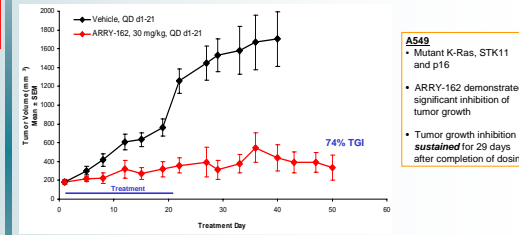
## Activity in Human CRC Xenografts – B-Raf Mutant



## Activity in BxPC3 Human Pancreatic Adenocarcinoma- No B-Raf or K-Ras Mutations



## Activity in A549 Human NSCLC- K-Ras Mutant



## ARRY-162: Summary

- Is a potent and selective inhibitor of MEK1/2
- Demonstrated broad anti-proliferative activity *in vitro* and *in vivo*
- Has well-behaved pharmacokinetic properties across species and possesses a good safety profile in preclinical studies (through chronic 6 month and 9 month GLP toxicology assessments)
- Is efficacious against K-Ras and B-Raf mutant tumors (A549, HT-29, COLO 205) as well as tumors harboring neither mutation (BxPC3) and inhibits tumor growth in numerous other xenograft models
- ARRY-162, alone and in combination (see poster #2414), is well-tolerated in preclinical xenograft models
- ARRY-162 demonstrated dose-related inhibition of tumor growth
- Tumor growth inhibition **sustained** at high dose for 7 days after completion of dosing

## Summary

- ARRY-162:
  - Is a potent and selective inhibitor of MEK1/2
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  - Has well-behaved pharmacokinetic properties across species and possesses a good safety profile in preclinical studies (through chronic 6 month and 9 month GLP toxicology assessments)
  - Is efficacious against K-Ras and B-Raf mutant tumors (A549, HT-29, COLO 205) as well as tumors harboring neither mutation (BxPC3) and inhibits tumor growth in numerous other xenograft models
  - ARRY-162, alone and in combination (see poster #2414), is well-tolerated in preclinical xenograft models
- ARRY-162 is currently in a Phase 1 dose escalation trial in patients with solid tumors