Investigation of the Growth Inhibitory Activity of the MEK Inhibitor ARRY-162 in Combination with Everolimus in a Variety of KRas and PI3K Pathway Mutant Cancers

Brian Tunquist, Tyler Risom, Debbie Anderson, Jennifer Garrus, Shannon Winski, Eli Wallace, Jim Winkler, Kevin Koch, Patrice Lee, Duncan Walker, and Stefan Gross

AACR 101st Annual Meeting 2010, Washington D.C.
Experimental and Molecular Therapeutics Section 29; Abstract #3855
Oncogenic mutations of certain receptor tyrosine kinases (RTK), Ras, and Raf frequently manifest in constitutive activation of MEK1/2 and ERK1/2 kinases.

This pathway has attracted much attention in the search for new chemotherapeutics, and small molecule inhibitors of MEK1/2 have been identified.

ARRAY-162 is a potent and selective inhibitor of MEK1/2:
Mutation of Raf, and to some extent, Ras, confers sensitivity to the MEK inhibitor AZD6244 (ARRY-142886).

Mutational activation of the PI3K pathway correlates with resistance.
Ras Activation of PI3K and Raf Pathways in Cancer

- Ras activates the PI3K and Raf/MEK/Erk signaling pathway.
- The PI3K pathway may compensate for the loss of Raf/MEK/Erk signaling.

frequently mutated in cancers
Would inhibition of Raf/MEK/ERK and mTor signaling pathways yield greater activity in the clinic through enhanced disruption of survival and proliferation signals?
Experimental Design

Cell Lines: 47 in total encompassing a wide range of tumor types and mutational status. Specifically, mutation of BRaf, Ras, PIK3CA, and PTEN genes were of interest.

Assay: Proliferation (Alamar Blue, DNA), 3-5 day duration

Compounds:
Everolimus: 10 nM (minimum concentration yielding maximum growth inhibition across all lines)
ARRY-162: 10, 30 & 100 nM (2x, 6x and 20x mechanistic cell IC\textsubscript{50})

Combination Activity Determination:
Bliss Method of Fractional Independence (Bliss, Cl. Ann Appl Biol. 1939; 26:585–615)
ARRY-162 and everolimus *in vitro* Combination Activity in Malme3M Cells

![Graph showing combination index (CI) values and percent of control (POC) for ARRY-162 and everolimus. Cl = 0.44.](image)

**Graph Legend:**
- **Synergism**
- **Additivity**
- **Independence/**
- **Antagonism**

**Graph Details:**
- **X-axis:** [ARRY-162] (μM) for ARRY-162 and [everolimus] (nM) for everolimus.
- **Y-axis:** Percent of control (POC).
- **Combination Index (CI):** 0.44
- Enhanced combinatorial activity in Ras or Raf mutant tumor cell lines:
  - majority of cell lines exhibit a CI value of less than 1
  - many of the cell lines exhibit synergy (CI < 0.8)
Further Stratification Based Upon PI3K Pathway Mutation

- Combination activity may not be enhanced with an additional mutation of PI3K, while tumors with a PTEN mutation may respond negatively.
- Experiments analyzing the effect of PTEN knockdown in sensitive cell lines are underway.
Does in vitro combination activity translate *in vivo*?

### Tumor Cell Lines (grouped by genotype)

- **SW620** colorectal cancer tumor cell line: KRas (G12V), CI = 1.2
- **NCI-H460** lung cancer tumor cell line: KRas (Q61H), PI3KCA (E545K), CI = 0.73
in vivo Comparison of SW620 and NCI-H460 Cell Lines

- *in vitro* combination activity is consistent with *in vivo* xenograft data.
- Decreased pErk levels by ARRY-162
- Unable to identify markers of sensitivity/resistance to the combination.
- No change seen in levels of Bcl-2, Bcl-X<sub>L</sub>, Bid, NOXA, or cleaved PARP (not shown).
- Regulation of Bim, p27 Kip1, and Rb proteins by MEK inhibitors has been reported.
- Unable to identify markers of sensitivity/resistance to the combination.
Summary

- ARRY-162 is a potent and selective inhibitor of MEK1/2
- Combination activity of ARRY-162 and everolimus may be enhanced by selection of tumors with specific genetic alterations:
  - Mutation of Ras or Raf is a potential marker of additivity/synergy
  - No clear impact from mutation of PI3K or PTEN
    - Possible exception is PTEN in wildtype cell lines
    - Investigations are underway to determine whether loss of PTEN is able to decrease combination activity in sensitive cells
- Combination activity \textit{in vitro} correlates with activity \textit{in vivo}
- Analysis of obvious pathway components has not revealed biomarkers of sensitivity/resistance to the combination thus far.
Preliminary Implications

- **Patient Selection:**
  - Patients with mutations in Ras or Raf may benefit from ARRY-162/everolimus combination treatment
  - Patients with mutation of PI3K may also benefit from this combination
  - Patients with a loss of function mutation in PTEN may not respond to this combination

- **Key indications with a high incidence of Ras or Raf mutations include:**
  - melanoma
  - NSCLC
  - colorectal cancer

- **Cell Autonomous vs. Tumor Microenvironment**
  - Additional benefit may be gained from effects of this combination on tumor hypoxia and metabolism.

- **ARRY-162 is currently in a Phase 1 study in patients with solid tumors.**