Dual inhibition of AKT and KIT is synergistic in Gastrointestinal Stromal Tumor

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Abstract

Purpose: The majority of gastrointestinal stromal tumors (GIST) harbor oncogenic mutations in the receptor tyrosine kinase KIT or in the platelet-derived growth factor receptor alpha (PDGFRA). Small molecule kinase inhibitors such as imatinib mesylate (IM) have significantly improved the clinical management of GIST by targeting these mutant receptors. Despite strong overall response rates to IM and other second-line targeted therapies, disease progression generally does occur in time. It is clear that certain mutations in KIT and PDGRA pathways provide a resistance mechanism to IM therapy. Therefore, inhibiting targets other than, or perhaps in addition to, traditional tyrosine kinases may provide additional therapeutic benefit in GIST. Both KIT and PDGFRA activate AKT and recent studies associate PI3-kinase/AKT pathway activity with the survival of IM-resistant GIST cell lines and tumors. In this study, we performed *in vitro* and *in vivo* experiments to assess the potential benefit of combining IM with an ArQule AKT inhibitor, ARQ 092. **Methods**: In order to evaluate *in vitro* drug sensitivity, a panel of IM-sensitive (GIST-T1, GIST882) and resistant (GIST-T1/829, GIST430) GIST cell lines were subjected to drug treatment for 72 hours before measuring viability with the Cell Titer Blue Viability Assay. Synergy between IM and ARQ092 inhibitor was quantified using the Chou-Talalay algorithm to calculate Combination Index (CI) values. CI values <1 are considered synergistic. *In vivo* studies evaluating ARQ 092 as a monotherapy and in combination with IM were performed using GIST-T1 and GIST430 xenograft models, as well as an IM-resistant, *KIT* exon 9 mutated GIST PDX model. **Results**: The 3:1 molar ratio of ARQ 092:IM demonstrated synergistic CI values in all four GIST lines. Immunoblot assays confirmed that drugs hit their intended targets (phospho-KIT, phospho-AKT) in each cell line following six-hour drug treatment. Interestingly, a significant decrease in the activation of a downstream signaling protein, p-S6, was observed in the combination-treated cells compared to cells treated with single agents. In addition, combination therapy provided significantly greater efficacy in both IM-sensitive and resistant xenograft models of GIST. **Conclusion**: These data provide strong preclinical justification for combining IM with an AKT inhibitor in both the front-line and second-line setting in GIST.

Biochemical potency and selectivity of ARQ 092

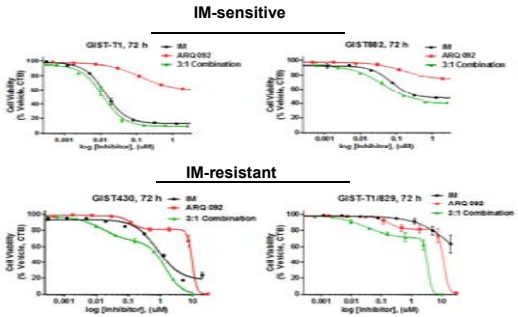
		Biochemical IC_{50} (nM)		
	Type	AKT1	AKT2	AKT3
ARQ 092	Allosteric	5.0	4.5	16
MK-2206	Allosteric	40.5	29.5	36.4
GDC-0068	ATP-competitive	2.0	27.0	6.3

Kinase Selectivity	
ARQ 092	
Kinase	IC_{50} (nM)
MARK4	129
MARK3	173
MARK1	180
DYRK2	386
IRAK1	806
Haspin	1160

Yu Y, Savage RE, Eathiraj S, Meade J, Wick MJ, Hall T, et al. (2015) Targeting AKT1-E17K and the PI3K/AKT Pathway with an Allosteric AKT Inhibitor, ARQ 092. PLoS ONE 10(10): e0140479. doi:10.1371/journal.pone.0140479

- The biochemical IC_{50} values for ARQ 092, MK-2206 and GDC-0068 were determined against full-length active forms of AKT 1, 2, and 3 (top).
- The biochemical IC_{50} values of ARQ 092 against 303 kinases were determined (Carna Biosciences) (bottom).

ARQ 092 and IM have synergistic effects on *in vitro* GIST cell growth



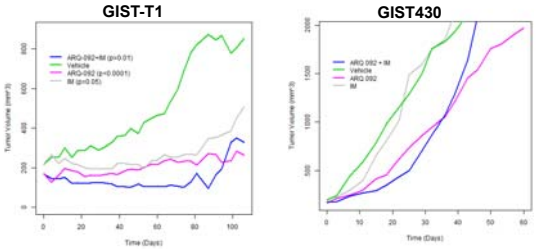
- Treatment with the ARQ 092 + IM combination at a 3:1 molar ratio resulted in enhanced sensitivity to the drugs in IM-resistant GIST cell lines (GIST430, GIST-T1/829).

CI Values (IC30) using CaluSyn (+/-) SEM).	
Cell Line	**3:1 ARQ 092:IM**
GIST-T1	0.56 ± 0.15
GIST882	0.40 ± 0.08
GIST430	0.38 ± 0.08
GIST-T1/829	0.32 ± 0.02

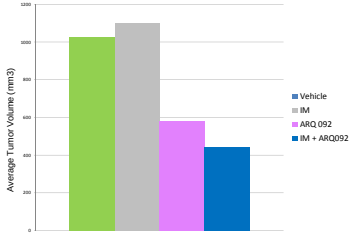
*Several molar ratios of the combinations were tested. The 3:1 (ARQ 092:IM) molar ratio was synergistic across all four GIST cell lines, consistent with our previous findings using the MK-2206 and IM combination in GIST cell and in vivo GIST studies.

(Zook P, Pathak HB, Belinsky M, Gersz L, Devarajan K, Zhou Y, Godwin AK, von Mehren M, and Rink, L. (2016) Combination of Imatinib Mesylate and AKT Inhibitor Provides Synergistic Effects in Preclinical Study of Gastrointestinal Stromal Tumor. Clin Cancer Res **DOI:** 10.1158/1078-0432.CCR-16-0529

Combination treatment reduces tumor growth *in vivo*

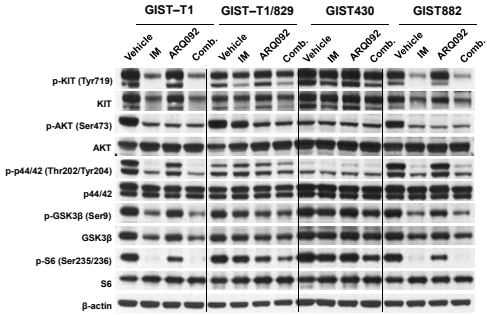


Statistically significant decreases in the rate of GIST-T1 tumor growth were observed due to treatment with IM (50mg/kg 5x/week, oral) (gray), ARQ 092 (75 mg/kg 3x/week, oral) (pink) and their combination (IM & ARQ 092 at monotherapy doses) (blue) compared to vehicle (green). GIST430 slope comparisons were not statistically significant, although at later time points statistically significant differences were observed. Tumor growth curves (tumor volume versus time) were computed for each treatment in the R statistical language (www.r-project.org).



4 week treatment of the fully characterized GIST7297 *KIT*^{exon9} p.A502_Y502dup PDX model with IM (50mg/kg 5x/week, oral) (gray), ARQ 092 (75 mg/kg 3x/week, oral) (pink) and their combination (IM & ARQ 092 at monotherapy doses) (blue),

Response markers of IM and ARQ 092



Conclusions and future directions

- ARQ 092 is a potent and selective allosteric AKT inhibitor.
- *In vitro* studies demonstrated drug synergy between ARQ 092 and IM at a 3:1 molar ratio in a panel of IM-sensitive and –resistant GIST cell lines.
- ARQ 092/IM combination therapy provided significantly greater efficacy in both IM-sensitive and resistant xenograft models of GIST.
- These data provide strong preclinical justification for testing the combination of IM with ARQ 092 in GIST in the clinical setting.