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

Phillip Zook[1], Martin Belinsky[1], Yi Yu[2], Giovanni Abbadessa[2], Terence Hall[2], Margaret von Mehren[1] and Lori Rink[1]

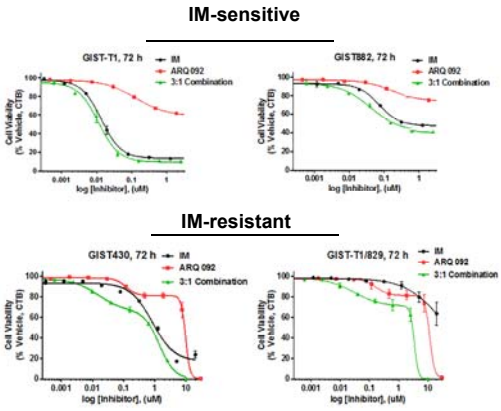
1 Molecular Therapeutics Program, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA

2 ArQule Inc., Burlington, Massachusetts, USA

Abstract

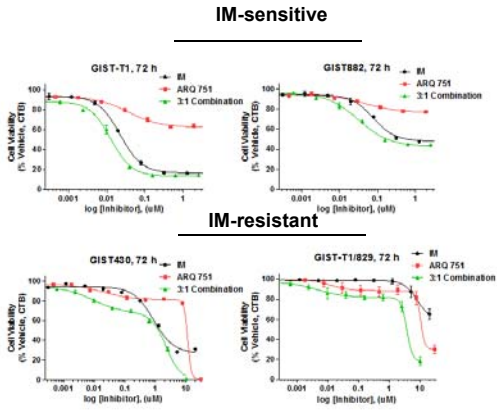
Purpose: The majority of gastrointestinal stromal tumors (GIST) harbor oncogenic mutations in the receptor tyrosine kinase KIT or in the platelet-derived growth factor receptor alpha (PDGFRA). Small molecule kinase inhibitors such as imatinib mesylate (IM) have significantly improved the clinical management of GIST by targeting these mutant receptors. However, 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. **Experimental Design**: Here, we performed experiments to assess the potential benefit of combining IM with an ArQule AKT inhibitor, either ARQ 092 or ARQ 751, in a panel of IM-sensitive (GIST-T1, GIST882) and resistant GIST cell lines (GIST-T1/829, GIST430). To evaluate *in vitro* drug sensitivity, cells were subjected to drug treatment for 72 hours before measuring viability with the Cell Titer Blue Viability Assay. Synergy between IM and each AKT inhibitor was quantified using the Chou-Talalay algorithm to calculate Combination Index (CI) values. CI values <1 are considered synergistic. **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. **Conclusion**: These data provide strong rationale for further testing of these combinations in GIST xenograft models.

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).

ARQ 751 and IM have synergistic effects on *in vitro* GIST cell growth



- Treatment with the ARQ 751 + IM combination at a 3:1 molar ratio resulted in enhanced sensitivity to the drugs in IM-sensitive and resistant GIST cell lines.

*Several molar ratios of the combinations were tested. The 3:1 (ARQ 092/ ARQ 751: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 of IM and AKT inhibitor displays synergism in *in vitro* GIST cell lines

CI values (IC_{30}) using CalcuSyn (+/-SEM).		
Cell Line	**3:1 ARQ 092 : IM**	**3:1 ARQ 751 : IM**
GIST-T1	**0.56 ± 0.15**	**0.36 ± 0.06**
GIST882	**0.40 ± 0.08**	**0.44 ± 0.03**
GIST430	**0.38 ± 0.08**	**0.15 ± 0.03**
GIST-T1/829	**0.32 ± 0.02**	**0.70 ± 0.06**

Biochemical potency and selectivity of ARQ 092 and ARQ 751

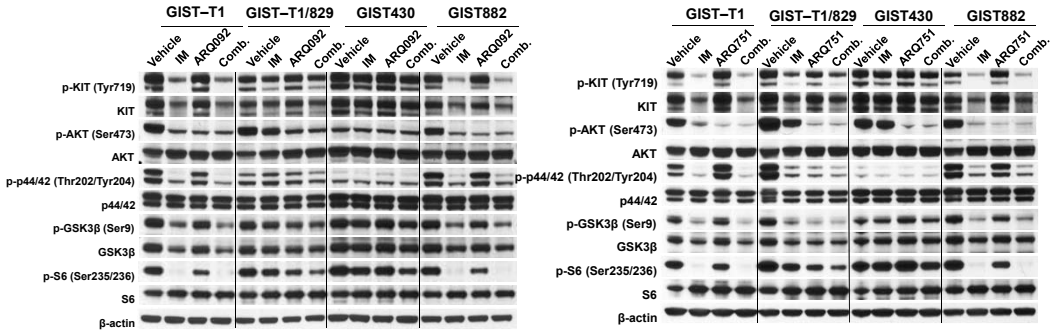
		Biochemical IC_{50} (nM)		
	Type	AKT1	AKT2	AKT3
ARQ 092	Allosteric	5.0	4.5	16
ARQ 751	Allosteric	0.55	0.81	1.3
MK-2206	Allosteric	40.5	29.5	36.4
GDC-0068	ATP-competitive	2.0	27.0	6.3

Kinase selectivity				
ARQ 092			ARQ 751	
Kinase	IC_{50} (nM)		Kinase	% inh. at 5 μM
MARK4	129		Blk(h)	40
MARK3	173		Tie2	33
MARK1	180		Haspin	30
DYRK2	386		Met	28
IRAK1	806		SGK3(119-end)	28
Haspin	1160		PASK	26
			Other 238 kinases	<25

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, ARQ 751, MK-2206, and GDC-0068 were determined against full-length active form of AKT1, 2, and 3 (top).
- The biochemical IC_{50} values of ARQ 092 against 303 kinases were determined (Carna Biosciences). The percent kinase inhibition of ARQ 751 was determined at a concentration of 5μM against a panel of 245 kinases.

Response markers of IM, ARQ 092 and ARQ 751



Conclusions and future directions

- ARQ 092 and ARQ 751 are potent and selective allosteric AKT inhibitors.
- *In vitro* studies demonstrated drug synergy between ARQ 092/ARQ 751 and IM in 3:1 molar ratio in a panel of IM-sensitive and -resistant GIST cell lines.
- We provide evidence for the initiation of preclinical studies evaluating the use of IM in combination with AKT inhibitors in *in vivo* models of GIST.