Evaluation of the activity of Rigosertib, an inhibitor of PI3K and MAPK pathways, in a preclinical model of colorectal cancer

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Background

Rigosertib (ON 01910) is an inhibitor of the PI3K and MAPK pathways and is currently being evaluated in clinical trials for pancreatic cancer and hematologic diseases. The PI3K/AKT and MAPK (RAS/RAF/MEK/ERK) signaling pathways have been identified to play a central role in the development and progression of CRC by modulating many different downstream targets that are essential for enhancing cellular survival and proliferation. Mutations in the PI3K (PIK3CA) and MAPK (RAS) pathways have been identified and their importance is well-established in oncogenesis and as drivers of tumor growth. The objective of this study is to determine the anti-proliferative and anti-tumor effects of rigosertib in a preclinical model of colorectal cancer (CRC).

Materials and Methods

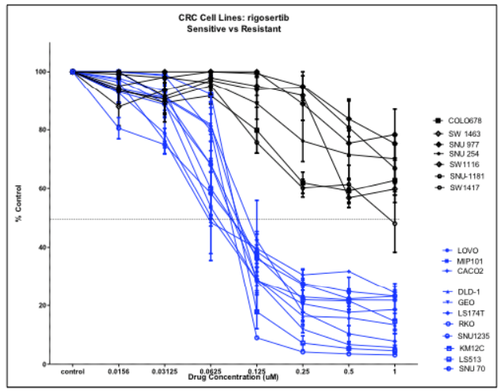
We used colorectal cancer cell lines and a patient-derived explant model to investigate the growth inhibitory activity of rigosertib. We treated 44 CRC cell lines with rigosertib for 72 hours and measured the effect on proliferation by a sulforhodamine B (SRB) assay. We treated 11 patient-derived CRC explants with rigosertib (250 mg/kg/day-daily) for 28 days to determine anti-tumor activity *in vivo*. A tumor growth index (TGI) ≤ 50% was considered sensitive.

Results

SRB Assay: 44 CRC cell lines

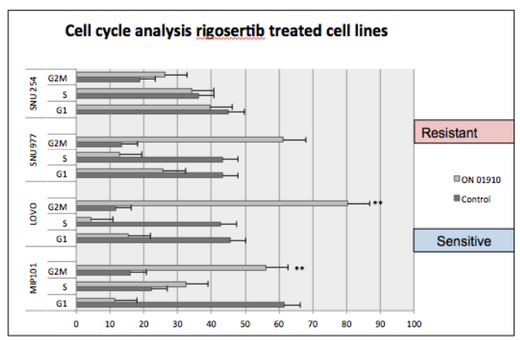
Cell Lines	IC-50	KRAS Exon 2	PIK3CA	APC
MIP 101	.0625 uM	MUT	MUT	
LOVO	.0625 uM	MUT	MUT	MUT
CACO2	.0625 uM	WT	WT	MUT
DLD-1	.125 uM	MUT	MUT	
LS174T	.125 uM	MUT	MUT	WT
GEO	.125 uM	MUT	WT	
RKO	.125 uM	WT	MUT	WT
SNU 1235	.125 uM	WT	WT	MUT
SNU 70	.125 uM	WT	WT	MUT
KM12C	.125 uM	WT	WT	
LS513	.125 uM	MUT	WT	WT
SW1417	1 uM	WT	WT	MUT
COLO678	n/a	MUT	WT	
SW1116	n/a	MUT	WT	MUT
SW1463	n/a	MUT	WT	MUT
SNU-977	n/a	WT	WT	MUT
SNU-1181	n/a	MUT		MUT
SNU 254	n/a	MUT	WT	MUT

Twenty-four CRC cell lines were sensitive to rigosertib with an IC_{50} < 0.1 µM. The cell lines MIP-101, Lovo, and Caco-2 were the most sensitive reaching an IC_{50} at 0.0625 µM. The Colo678, SW 1116, SW 1463, SNU-977, SNU-1181, and SNU 254 cell lines were resistant to rigosertib with an IC_{50} > 1µM. There was no association between mutational status in PIK3CA or RAS and sensitivity to rigosertib.

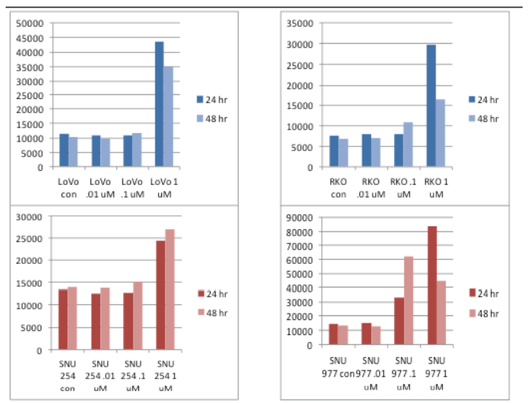


Krishan's Cell Cycle Analysis:
2 sensitive vs 2 resistant cell lines

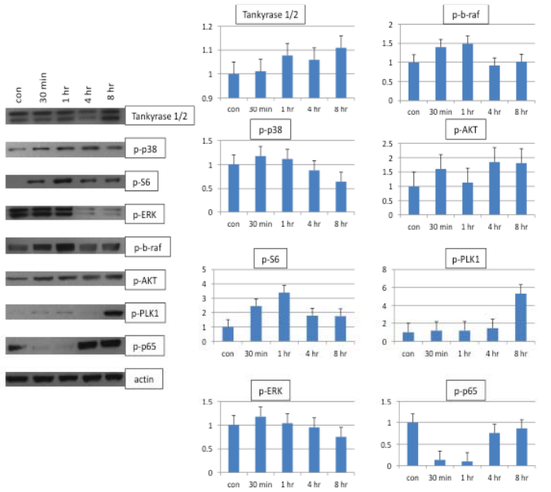
There are significant differences in cell cycle progression from control to treatment with rigosertib, with treated cells arresting in G2/M.



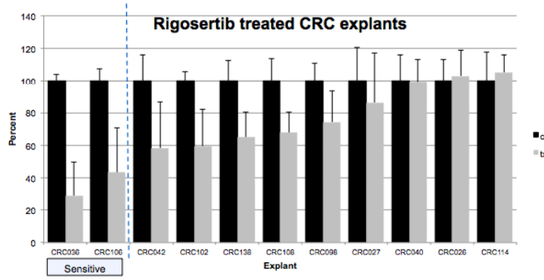
Caspase 3/7 Assay:
2 sensitive vs 2 resistant cell lines



Protein Quantification: Western Blot on RKO, a sensitive CRC cell line to rigosertib



Colorectal Tumor Explants:
11 Explants treated with rigosertib



Specimen ID	KRAS	PIK3CA	APC	CTNNB1	NRAS	BRAF
CRC-026	WT	WT	Mut	WT	Mut	WT
CRC-027	Mut	WT	Mut	WT	WT	WT
CRC-036	Mut	WT	Mut	WT	WT	WT
CRC-040	Mut	Mut	WT	WT	WT	WT
CRC-042	Mut	Mut	WT	Mut	WT	WT
CRC-098	Mut	Mut	Mut	WT	WT	WT
CRC-102	Mut	WT	WT	WT	WT	WT
CRC-106	WT	WT	WT	WT	WT	WT
CRC-108	Mut	WT	WT	WT	WT	WT
CRC-114	WT	WT	Mut	WT	WT	Mut
CRC-138	Mut	WT	Mut	WT	WT	WT

Conclusion

We found rigosertib to be a potent anti-proliferative agent in vitro. Of the 11 explants treated, 2 are sensitive to treatment with rigosertib.

Acknowledgements

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