Rigosertib, a novel inhibitor of Ras signaling, overcomes azacitidine resistance in acute myeloid leukemia cell lines

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ABSTRACT

Background: Azacitidine (AZA) and decitabine (DEC) are FDA approved hypomethylating agents (HMAs) used for treating MDS and acute myeloid leukemia (AML). Resistance to HMAs develops in most patients and is poorly understood. New approaches to addressing resistance and new treatment options following HMA failure are needed.

Introduction: MDS and AML patients are categorized as primary AZA-resistant or AZA-sensitive who become resistant over time with a response rate of only 17%. Once MDS patients fail AZA treatment, median survival is less than 6 months. Rigosertib is a novel agent that inhibits multiple signaling pathways, primarily by interfering with Ras/Raf binding and down-regulating pathways (including PI3-K and PLK-1), resulting in apoptosis. Rigosertib has been shown in vitro to act synergistically with AZA. Rigosertib is currently in trials as a single IV agent in MDS patients who have relapsed or are refractory to HMAs as an oral formulation in combination with AZA in patients with MDS/AML.

Purpose: To measure the growth inhibition activity of rigosertib as a single agent or in combination against AZA-resistant AML cells and to follow dose- and time-dependent changes in survival and apoptosis pathways.

Materials and Methods: Two AZA-resistant cell lines were studied, along with their sensitive parental counter parts. 96-hr dose-response assays were performed using rigosertib as a single-agent or in combination with AZA. Cells were also tested for cross-resistance toward a number of chemotherapy agents, including DEC.

Results: HL-60 parental versus resistant cells showed that resistant cells were 10-fold resistant toward AZA but not cross-resistant toward rigosertib (GI50 of 0.25 µM parental vs. 0.15 µM resistant). Likewise, the resistant THP-1 cells were 24-fold resistant to AZA but still sensitive toward rigosertib (GI50 of 0.0625 µM parental vs. 0.175 µM resistant). In the HL-60/AR cells, cross-resistance was observed toward DEC but not cytarabine, mitoxantrone, doxorubicin, vincristine or cisplatin. THP-1 parental cells were intrinsically resistant toward DEC and cytarabine, and there was no cross-resistance in either THP-1 cell line toward the other agents tested. Combination studies showed that rigosertib acts additively or synergistically with AZA in a schedule-dependent manner. Rigosertib treatment of the AZA-resistant cell lines resulted in down-regulation of survival pathways and induction of apoptosis.

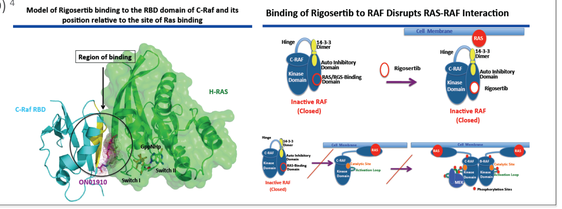
Conclusion: These results provide the rationale for using rigosertib as a treatment for MDS and AML patients who develop AZA resistance.

BACKGROUND

Clinical Relevance: Onconova is currently conducting a Phase 2 clinical trial with oral rigosertib in combination with the hypomethylating agent, azacitidine, in higher-risk MDS and AML patients (NCT# 1926587). Positive Phase 1 results from this study were presented at the 2014 ASH Conference (Navada et al., Abstract 3252)[1]. This trial is based on non-clinical findings demonstrating the synergistic anti-leukemic activity of the combination of rigosertib and azacitidine (Skidan et al., AACR 2006 Abstract 1310[2].

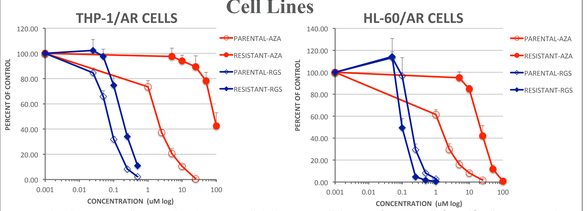
Cell Lines: The Azacitidine resistant cells were developed by growth and continual selection in medium containing azacitidine (10uM) and kindly provided by Dr. Tadashi. Nagai[3].

Model of Mechanism of Action of Rigosertib: Rigosertib Binds to Ras Binding Domains (RBD) [4]



RESULTS

Fig 1: Rigosertib Is Cytotoxic Towards Azacitidine Resistant Cell Lines



Parental and resistant cells were plated into 96 well dishes at a a cell density of 10,000 cells/0.1ml/well in RPMI medium. The cells were treated with increasing concentrations of freshly prepared 5-azacytidine or Rigosertib (RGS) and viability was determined 96 hours later. The data is plotted as the average number of viable cells as a percent of vehicle control (+/-) SD.

Table 1: Single-Agent Cytotoxicity in AZA Sensitive and Resistant Cell Lines

COMPOUND	CELL LINE	GI50 (uM)*	Fold Resistant
5-AZACYTIDINE	HL-60 PARENTAL	2	10
	HL-60/AR**	20	
RIGOSERTIB	HL-60 PARENTAL	0.25	0.6
	HL-60/AR	0.15	
5-AZACYTIDINE	THP-1 PARENTAL	2.5	24
	THP-1/AR	60	
RIGOSERTIB	THP-1 PARENTAL	0.0625	2.0
	THP-1/AR	0.125	

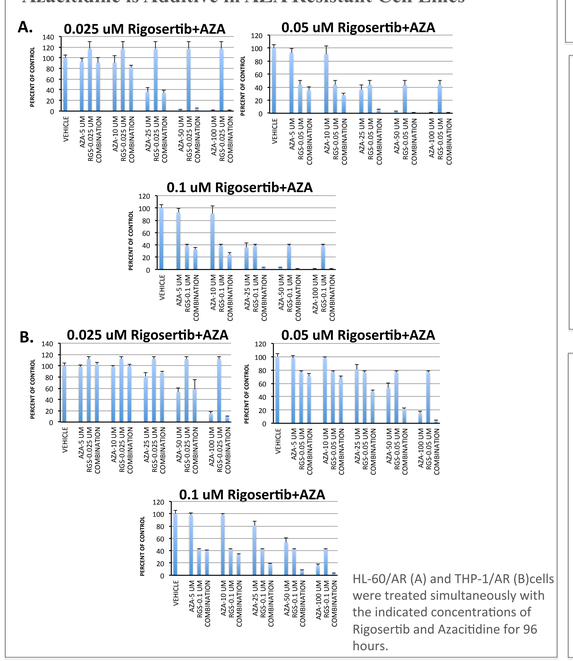
* The GI50 value is the concentration required for 50% inhibition as compared to vehicle control. The value is the average of two independent experiments (N=8)
** AR represents the Azacitidine Resistant clones.

Table 2: GI50 Values and Fold Resistance of Various Chemotherapeutic Agents

Compound	HL-60	HL-60/AR**	FOLD RES	THP-1	THP-1/AR	FOLD RES
Decitabine	1*	25	25	10	>100	>10
Cytarabine	0.015	0.05	3	0.75	2	3.3
Mitoxantrone	0.002	0.002	0	0.0075	0.005	0.7
Doxorubicin	0.025	0.04	1.6	0.04	0.075	1.9
Vincristine	0.002	0.002	0	0.0003	0.001	3.3
Cisplatin	1.5	1.5	0	3	4	1.3

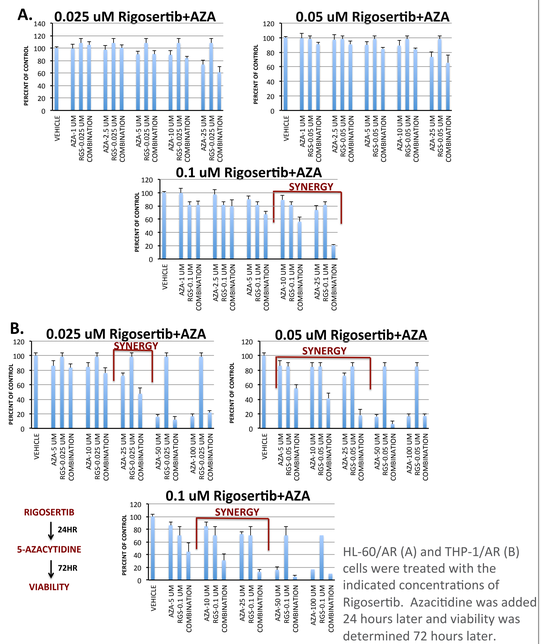
* The GI50 values are noted as uM concentrations.
** AR represents the Azacitidine Resistant clones.

Fig 2: Simultaneous Administration of Rigosertib and Azacitidine is Additive in AZA Resistant Cell Lines



HL-60/AR (A) and THP-1/AR (B)cells were treated simultaneously with the indicated concentrations of Rigosertib and Azacitidine for 96 hours.

Fig 3: Rigosertib Acts Synergistically With Azacitidine When Given Prior To Azacitidine



HL-60/AR (A) and THP-1/AR (B) cells were treated with the indicated concentrations of Rigosertib. Azacitidine was added 24 hours later and viability was determined 72 hours later.

Fig 4: Rigosertib Induces Apoptosis of AZA Resistant Cell Lines: 24HR Exposure

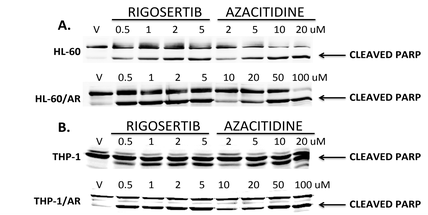


Fig 5: Rigosertib Interferes with the Ras Effector Pathways, PI3K and MAPK, in AZA Sensitive and Resistant Cell Lines: 24HR Exposure

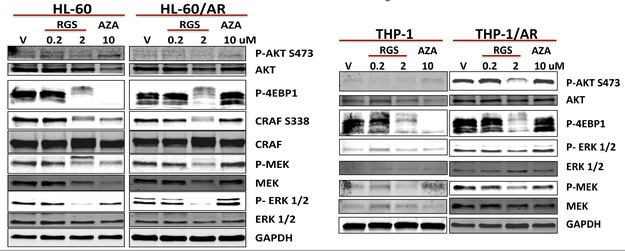
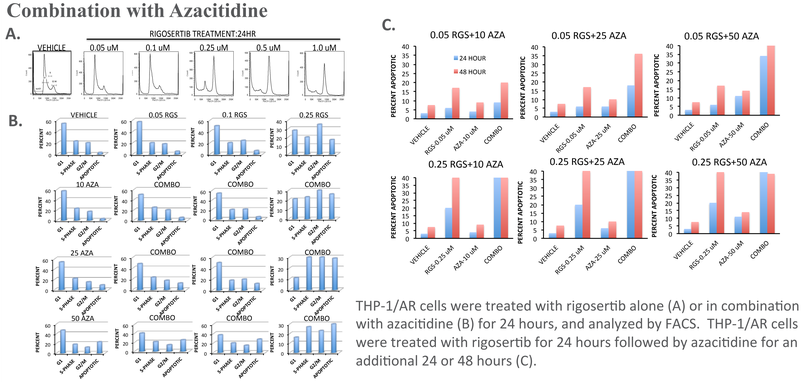
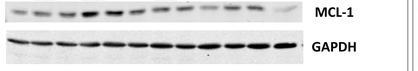


Fig 6: Cell Cycle Analysis of THP-1/AR Cells Treated with Rigosertib Alone and In Combination with Azacitidine



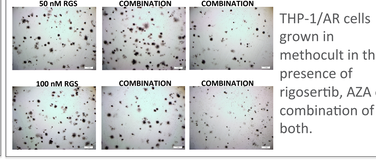
THP-1/AR cells were treated with rigosertib alone (A) or in combination with azacitidine (B) for 24 hours, and analyzed by FACS. THP-1/AR cells were treated with rigosertib for 24 hours followed by azacitidine for an additional 24 or 48 hours (C).

Fig 7: Rigosertib and AZA Treated THP-1/AR Cells, Synergistic Activation of Apoptosis



THP-1/AR cells were treated with Rigosertib for 24 hrs and then treated with Azacitidine for an additional 24 hours. Total cellular protein was used for western blotting.

Fig 8: Rigosertib and Aza Combination Treatment: 3D Growth of THP-1/AR Cells



THP-1/AR cells grown in methocult in the presence of rigosertib, AZA or a combination of both.

CONCLUSION

- Rigosertib is cytotoxic to both azacitidine resistant cell lines which show cross resistance to decitabine and cytarabine. HL-60/AR and THP-1/AR have been shown to express mutant uridine-cytidine kinase 2 (UCK2)[3] gene leading to AZA resistance but other unknown mechanism(s) leading to cross resistance towards decitabine and cytarabine do not allow survival following rigosertib treatment.
- Rigosertib (24hr) treatment induces apoptosis in both parental and resistant cell lines.
- Rigosertib Interferes with the Ras Effector Pathways, PI3K and MAPK, in AZA Sensitive and Resistant Cell Lines
- Simultaneous treatment with rigosertib and azacitidine results in additive activity while treatment with rigosertib 24hr prior to azacitidine treatment is synergistic.
- Cell cycle analysis shows that rigosertib treatment induces a dose dependent mitotic block and induction of apoptosis of resistant cell lines and combination studies confirm the sequence dependent additive and synergistic activity of combining rigosertib and azacitidine.
- **These results provide the rationale for using rigosertib as a treatment for MDS and AML patients who develop AZA resistance.**

REFERENCES

1. Navada S.C, Garcia-Manero G, Wilhelm F, Hearn K, Odchimar-Reissig R , Demakos E.P, Alvarado Y, Daver N, DiNardo C.D, Konopleva M, Borthakur G, Azarnia N, Silverman L.R. (2014) A Phase I/II Study of the Combination of Oral Rigosertib and Azacitidine in Patients with Myelodysplastic Syndrome (MDS) or Acute Myeloid Leukemia (AML) Blood 124, Abstract 3252.
2. Skidan I, Zinzar S, Holland J.F, Reddy R, Reddy E.P, and Silverman L. R. (2006) Toxicology of a novel small molecule ON1910Na on human bone marrow and leukemic cells in vitro Cancer Res 66, Abstract 1301
3. Sripayap P, Nagai T, Uesawa M, Kobayashi H, Tsukahara T, Ohmine K, Muroi K, Ozawa K. (2014) Mechanisms of resistance to azacitidine in human leukemia cell lines. Exp Hematol. Apr;42(4):294-306.
4. Divakar S, Vasqez-Del Caprio R, Baker S.J, Reddy M.V.R, Ritt D.A, Morrison D.K, Reddy E.P. 2014 Targeting the Ras-Binding domain of RAS effector proteins by a small molecule inhibitor, Rigosertib. Cancer Res 74, Abstract: LB-108.

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