Rigosertib (RIG) modulates MAPK and hematopoiesis signaling and synergizes Azacitidine (AZA) altering viral mimicry pathway in myelodysplastic syndrome (MDS)

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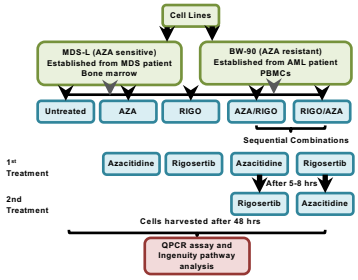
BACKGROUND

Myelodysplastic syndrome (MDS) is a heterogeneous stem cell disorder characterized by hyperproliferative bone marrow (BM) and peripheral blood cytopenias involving one or more lineages. Azacitidine (AZA), a hypomethylating agent (HMA) is consider 1st line therapy for higher-risk disease. About 50% of MDS patients (pts) respond to AZA, with a median response of 14-24 months. For pts responding to AZA, most either relapse or progress with worsening BM failure (secondary resistance) and have a median survival of 4 to 6 months. Both primary and secondary resistance remains a significant challenge and results in poor survival. We previously reported that Rigosertib (RIGO), a ras-mimetic identified as a novel anti-cancer drug, inhibits cell cycle progression, induces apoptosis of cancer cells and acts as a histone deacetylase inhibitor with chromatin modifying activity. In a Phase I/II study the combination of RIGO and AZA produced an overall response rate of 85% in pts who were HMA naive and 54% in HMA failures (Navada et al. ASH 2018). The ability to reverse the clinical resistance phenotype is a novel observation with clinical implications. In this study, we investigated the effect of AZA and RIGO alone or in sequential combination (SC) on MDS-L cell line to identify the mechanism of action of the drugs.

METHODOLOGY



Methods: Total RNA was extracted from AZA, RIGO, AZA/RIGO or RIGO/AZA treated MDS-L and BW-90 cells according the manufacturer's recommendations (Life Technology). c-DNA was prepared and Q-PCR assays were performed using RT profiler PCR arrays (Qiagen) as per manufacturer's instruction. Fold change was determined using Qiagen data analysis software and further pathway analysis for the differentially expressed genes was performed using Ingenuity Pathway Analysis software.

RESULTS

Table 1. List of differentially expressed genes in MDS-L and BW-90 cells treated with AZA, RIGO, AZA/RIGO and RIGO/AZA.

	MDS-L				BW-90			
Gene	AZA	RIGO	AZA/ RIGO	RIGO/ AZA	AZA	RIGO	AZA/ RIGO	RIGO/ AZA
Interferon Signaling								
CRLF2		2.73						
CSF3R			-2.20	-2.06				
IFI27						2.61		
IFI44		-2.63	-2.60	-2.16				
IFI44L						-2.01		
IFI6			-2.07					
IFIT1						-2.28		
IFNA6		3.92	5.67	2.16		2.22		
IL10RA			2.38	2.50				
IL4R			2.08	2.10				
IL5RA		-2.61	-2.91	-2.69				
IRF2BP1	-2.57				3.09			
Hematopoiesis signaling								
BLNK							-2.26	
CD3D			2.37					
CD4		-2.85	-3.34	-2.96				
CD44		3.26	2.62	3.46				
CD80				2.19				
CD86		-2.02						
CBFB							-2.05	
CHST15	2.56		2.82	3.60			-2.05	
CSF2							3.28	-2.13
GATA1							-2.21	-2.08
FUT10				2.03				
HDAC9							2.51	
IL25							-2.06	
NHBA		4.04	8.06	7.43				
MMP9		-2.65		-2.45				
SFXN1		2.07						
Epigenetic signaling								
DNMT3A		2.02	2.01					
KMT2C				2.17				
SMYD3		2.15						
TNFSF11						2.14		
AURKA					-2.24	-2.43		
MAPK signaling								
BRAF							-2.01	
CCNA1		3.08	3.16	2.89				
CCND1		2.24	3.16	2.43				
CDKN1A			2.00					
COL1A1						2.06		
EGR1		2.24	2.24					
HSPB1		3.31	2.90	2.92				
JUN		4.43	5.32	4.50		2.88		
MAPK6		2.05	2.18	2.01				
MAPKAPK2		2.23	2.16	2.15				
MOS				2.79				
MST1		-2.25	-2.09					

Figure 1. The pathways affected in MDS-L and BW-90 cell line upon treatment with AZA and RIGO ether alone or in sequential combinations.

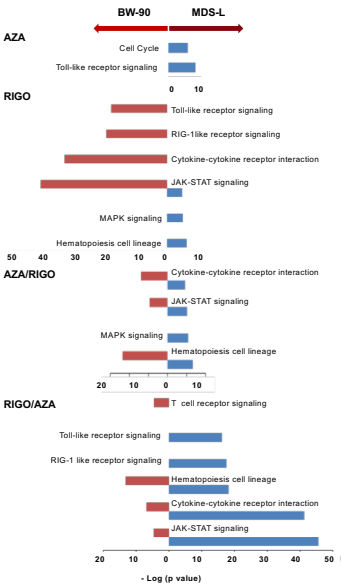


Figure 2. The variation in RIG-1 like receptor signaling in MDS-L and BW-90 cell lines upon treatment with AZA and RIGO ether alone or in sequential combinations.

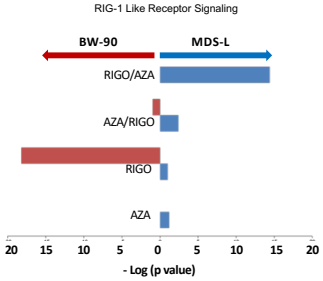
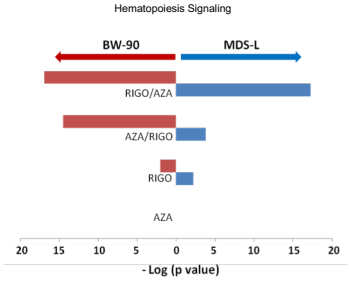


Figure 3. The variation in Hematopoiesis signaling in MDS-L and BW-90 cell line upon treatment with AZA and RIGO ether alone or in sequential combinations.



SUMMARY

- Anti-viral response, RIG-1 like receptor signaling pathway was the most affected pathway in RIGO/AZA treated MDS-L cells and RIGO treated BW-90 cell.
- Hematopoesis signaling was mostly affected in cells sequentially treated with AZA/RIGO or RIGO/AZA in both MDS-L and BW-90 cell lines. However, it was greatly impacted in RIGO/AZA treatment in both cell lines. The genes involved in Hematopoiesis signaling pathway genes were only upregulated in RIGO/AZA treated MDS-L cells only.

CONCLUSIONS

- These results indicate that the MDS-L and BW-90 cell lines which are very distinct in nature with respect to Azacitidine sensitivity shows contrasting results when treated with RIGO/AZA.
- Unique pathway signature were identified based on drug sequence and individual cell lines.
- The RIG-1 like receptor signaling pathway is uniquely upregulated only by RIGO/AZA sequence.
- RIGO/AZA upregulates hematopoiesis signaling by greater than 2 fold with RIGO alone or AZA/RIGO SCs in MDS-L cells.
- Further in-depth investigation is needed to understand the role of anti-viral sensing pathway on hematopoiesis in patients who are resistant to hypomethylating agents and are benefitted by combination of RIGO and AZA.

REFERENCES

Navada *et al.* Phase 2 Expansion Study of Oral Rigosertib Combined with Azacitidine (AZA) in Patients (Pts) with Higher-Risk (HR) Myelodysplastic Syndromes (MDS): Efficacy and Safety Results in HMA Treatment Naïve & Relapsed (Rel)/ Refractory (Ref) Patients. Blood, December 2018 ASH abstract.

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