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The Sequenced Combination of Rigosertib and Azacitidine Has Modulatory Effects on CXCL8, RIG-I like Receptor and Wnt/β-Catenin Signaling and Downstream Hematopoiesis Pathways in an in-vitro Model of the Myelodysplastic Syndrome.

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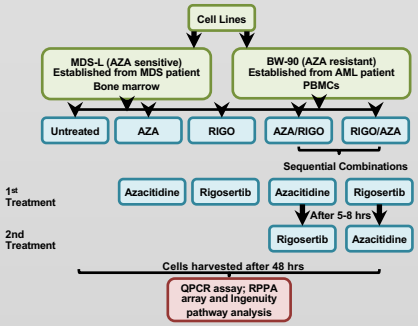
BACKGROUND

Myelodysplastic syndrome (MDS) is characterized by ineffective hematopoiesis and multiple cytopenias. Azacitidine (AZA), a hypomethylating agent (HMA), the standard therapy for higher-risk MDS patients (pts), improves hematopoiesis in 50% of MDS pts, with a median response of 14-24 months. Those pts who initially respond to AZA either relapse or progress with bone marrow failure and have a median survival of 4 to 6 months. Both primary and secondary resistance is a significant challenge and results in poor survival. Rigosertib (RIGO), a small molecule Ras mimetic, as a single agent improved hematopoiesis in 15% of MDS pts who had failed a prior HMA. In vitro data of synergy of RIGO combined with AZA that was sequence dependent (Skiddan et al. AACR 2006), led to a Phase I/II study of the combination of RIGO/AZA and demonstrated an overall response rate of 90% in HMA naïve and 54% in HMA failures pts (Navada et al. ASH 2018). Restoration of functional hematopoiesis in response to treatment with AZA when combined with RIGO in pts, who had failed an HMA, is an unique observation in overcoming the HMA clinical resistance phenotype. In this study, we investigated the molecular mechanism/ pathways impacted as a result of AZA and RIGO treatment either alone or in sequential combination (SC) on MDS-L and BW-90 cell lines.

METHODOLOGY



Methods: Total RNA was extracted from AZA, RIGO, AZA/RIGO or RIGO/AZA treated MDS-L and BW-90 cells according to 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. Protein validation was performed by Reverse phase protein array (RPPA) at MD Anderson, Texas. Pathway analysis for the differentially expressed genes/ proteins was performed using Ingenuity Pathway Analysis software.

RESULTS

Figure 1. The graph represents the pathway that are predominantly affected in MDS-L and BW-90 cells, RIGO and AZA SC shows maximum impact on all the pathways in MDS-L cells whereas RIGO alone impacts RIG-I like receptor signaling and T cell exhaustion signaling in BW-90 cells

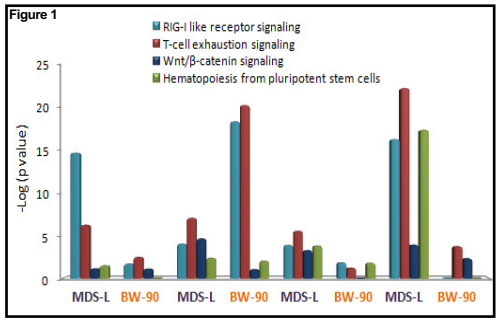


Figure 2. The expression of CXCL8 gene was elevated by 7-9 fold by RIGO treatment either alone or in SC with AZA as compared to controls in MDS-L cells.

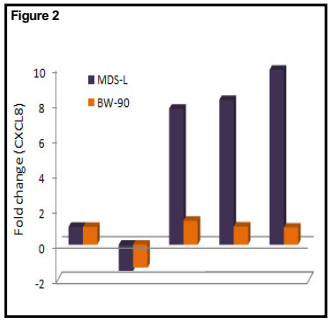


Figure 3. Heat map shows the differential protein expression on treatment with AZA and RIGO alone and their SCs in BW-90 and MDS-L cell lines by RPPA analysis.

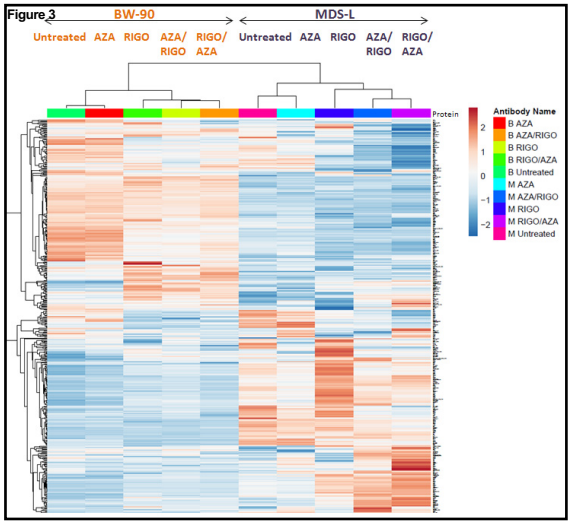


Figure 4. The expression of Jun and CD44 at mRNA level was observed to be elevated in response to RIGO alone and SCs in MDS-L cell lines .

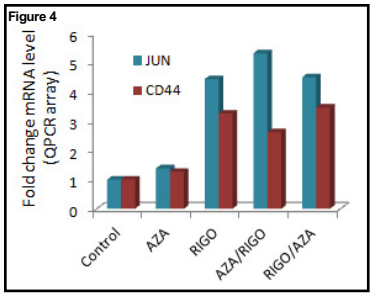
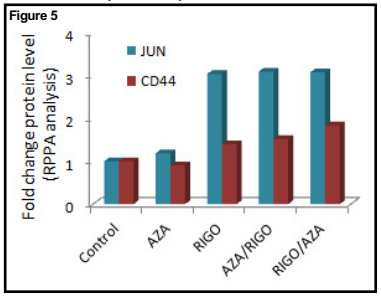


Figure 5. The expression of Jun and CD44 at protein level was observed to be elevated in response to RIGO alone and SCs in MDS-L cell lines by RPPA analysis.



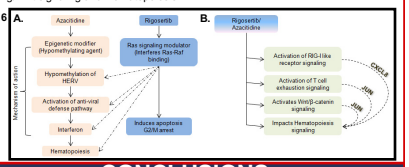
SUMMARY

• RIG-I like receptor (RLR) signaling (anti-viral defense pathway), T cell exhaustion signaling, Wnt/β-catenin signaling and hematopoiesis pathway were the most impacted pathways in MDS-L cells treated with RIGO/AZA combinations compared to other treatments. However, RIGO alone induces the dysregulation of RIG-I like receptor signaling and T cell exhaustion signaling in BW-90 cells.

• CXCL8 is a RLR signaling responsive gene and is also one of the genes which were observed to be involved in hematopoiesis signaling identified by pathway enrichment analysis. Interestingly, its expression was observed to be elevated in RIGO and SCs by 7-9 fold compared to untreated MDS-L cells.

• Wnt/β-catenin pathway was predicted to be specifically activated in MDS-L cells with SCs. Both QPCR and RPPA results demonstrated activation of Wnt/β-catenin signaling pathway in response to RIGO alone and the combination with AZA. Importantly, expression of two genes Jun (proto oncogene) and CD44 (Wnt target gene) that are associated with the Wnt/β-catenin signaling pathway were upregulated at both mRNA and protein level which suggests a crucial role of RIGO in wnt signaling.

Figure 6. Pictorial representation of the pathway that are associated with AZA and RIGO alone A. A hypothesized model; (-) solid line shows about the pathway that is known to be activated based on the review of literature, (---) dashed line shows the interaction that is not known yet. B. The pathways that were impacted by the RIGO/AZA combination which indicate there may be some correlation between RIG-I signaling/T cell exhaustion signaling/Wnt signaling and Hematopoiesis.



CONCLUSIONS

These result indicate that RIGO may have impact on hematopoiesis signaling via either RLR signaling or Wnt signaling. Further studies are underway to determine the effects of these signaling pathways on improving hematopoiesis both *in vitro* and *in vivo* in the HMA clinical resistance setting to identify potential therapeutic targets to reverse bone marrow failure in pts with HMA resistance.

REFERENCES

•Skiddan et al. Toxicology of a novel small molecule ON1910Na on human bone marrow and leukemic cells in vitro. AACR Abstract 1310, April 2006; 47:309.

•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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