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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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 (RGO), 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 RGO combined with AZA that was sequence dependent (Skiddan et al. AACR 2003), led to a Phase III study of the combination of RGO/AZA and demonstrated an overall response rate of 90% in HMA naïve and 54% in HMA failures pts (Navada et al. ASCO 2015). Restoration of functional hematopoiesis in response to treatment with AZA when combined with RGO 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 RGO treatment either alone or in sequential combination (SC) on MDS-L and BW-90 cell lines.

**RESULTS**

- **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.

**CONCLUSIONS**

These results indicate that RGO 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**


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**METHODOLOGY**

- **Methylation array** and **signaling pathway analysis**
- **Cell Lines**
  - MDS-L (AZA treated) (MDS-L/ARA CCRF-CEM; EMD: 154765)
  - MDS-L (AZA resistant) (MDS-L/AR 32D; EMD: 193948)
  - BW-90 (AZA resistant) (BW-90/AR 32D; EMD: 194011)
  - BW-90 (Bone marrow; al)

- **1st Treatment**
  - Untreated
  - AZA
  - RGO
  - AZA/RGO

- **2nd Treatment**
  - Cells harvested after 46 hrs
  - AZA
  - RGO
  - AZA/RGO

- **Methods:** Total RNA was extracted from AZA, RGO, AZA/RGO or RGO/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 R1 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 (RPQA) at MD Anderson, Texas. Pathway analysis for the differentially expressed genes/ proteins was performed using Ingenuity Pathway Analysis software.