Effects of Rigosertib (RIGO) Alone or in Combination with Azacitidine or Vorinostat on Epigenetic Reprogramming of CD34+ Cells in the Myelodysplastic Syndrome

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Background
Azacitidine (AZA) is the standard of care for patients (pts) with higher-risk MDS, however, only 50% of pts respond and the majority will relapse within 2 years. All pts ultimately fail treatment due to primary or secondary resistance. Rigosertib (RIGO) is a "ras mimetic" agent that binds to the Ras Binding Domain of RAF kinases and inhibits the RAS-RAF-MEK and the PI3K pathways. Initial results of an ongoing Phase I/II study with RIGO combined with AZA, in pts with MDS demonstrated a response rate of 76% overall; 62% in pts following hypomethylating agent (HMMA) failure and 85% in HMA naïve pts (Navada et al. EHA, 2017).

Rigosertib (RIGO) modulates HDACs (class I, II and IV) and DNM1 differentially in cell specific manner. MDS-L and BW90 cells were treated with AZA, RIGO, AZA/RIGO or RIGO/AZA for 48 hrs and Q-PCR using SybrGreen was performed. Fold change in relative transcript expression levels of HDACs and DNM1 genes in MDS-L and BW90 are given below

<table>
<thead>
<tr>
<th></th>
<th>CD34+</th>
<th>ALDH</th>
<th>Pluripotency Genes (SOX2; OCT4; NANOG; LDB3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rigosertib</td>
<td>No increase</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Azacitidine</td>
<td>3.8 x increase</td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td>Vorinostat</td>
<td>4 x increase</td>
<td>Decrease</td>
<td></td>
</tr>
<tr>
<td>Rigosertib + No increase</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Azacitidine</td>
<td>3.8 x increase</td>
<td>Decrease</td>
<td></td>
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Method
In vitro Study. We investigated the in vitro effects of RIGO combined with AZA on two cell lines: AML (BW90), MDS (MDS-L) cells and p bone marrow samples obtained prior and after 1 cycle of AZA and RIGO. MDS-L and BW90 cells were initially primed in serum-free StemLineL (Sigma-Aldrich) media overnight and treated with AZA, RIGO, AZA/RIGO or RIGO/AZA for 48 hrs.

Q-PCR assay. Total RNA was extracted from AZA or RIGO treated MDS-L and BW-90 cells and pts BM samples, cDNA was prepared and Q-PCR assays were performed using Syber Green.

Histone post-translational modifications assay. To identify cell populations with high and low levels of active (H3K4me2, H3K9ac and H3K18ac) and repressive (H3K27me3, H3K27me2 and H3K4me3) histone marks in CMA treated cells were stained with mAbs according to the manufacturer’s instructions (Cell Signaling Technology) and analyzed by using BD FACSCanto™ II Flow Cytometer.

Western blot. Whole-cell extracts were prepared from MDS-L and BW-90 cells after treatment with various drugs either alone or in combination for 48 hrs. Total cellular proteins were separated by SDS-PAGE and transferred by IBlot (Invitrogen). The Western-blot membranes were probed with mAbs against proteins from AKT, Cell cycle, Cdc25s signaling pathway and β-actin; Cell Signaling Technology) and developed using a chemiluminescence as per manufacturer’s instructions.

Results
Flow cytometry reveals the existence of different levels (Lo-low and HI-high) of histone H3 lysine-4, lysine-27 methylation (H3K4me2, H3K4me3, H3K27me2 and H3K4me3) and histone K3 lysine-18 and lysine-9 acetylation (H3K18ac and H3K9ac) following treatments with AZA, RIGO, AZA/RIGO and RIGO/AZA. On each panel, the bar graph shows quantification of the percentage of MDS-L cells representing Lo and HI distribution of various histone marks treated with AZA, RIGO, AZA/RIGO or RIGO/AZA for 48 hrs.

Effect of RIGO alone or in combination with AZA on cell cycle checkpoint proteins, apoptosis and AKT cell signaling pathway. Western blot analysis was performed on AKT signaling pathway proteins following 48 hrs of treatment with AZA, RIGO or AZA/RIGO/RIGO/AZA. β-Actin expression as control.

Western blot analysis revealed that RIGO alone or in combination with AZA were more effective in downregulating AKT signaling and cell cycle related proteins.

Conclusion
The epigenetic events modulated by RIGO in combination with either AZA or Vorinostat (VOR) led to:
- Global histone PTMs
- Differential PI3K association with active histone marks,
- Epigenetic reprogramming of pluripotency genes
- Expansion of primitive HSPC
- Downregulation of the PI3K/AKT pathway and cell cycle checkpoint proteins.

These epigenetic effects of RIGO on chromatin alterations lead to HSPC reprogramming.

These preclinical models suggest potential novel clinical strategies to improve outcomes for patients with higher-risk MDS.

References