

# Rigosertib (RIG) in combination with Azacitidine (AZA) modulates epigenetic pathways In Vitro and In Vivo and can overcome clinical resistance to hypomethylating agents (HMA) in Myelodysplastic Syndromes (MDS)

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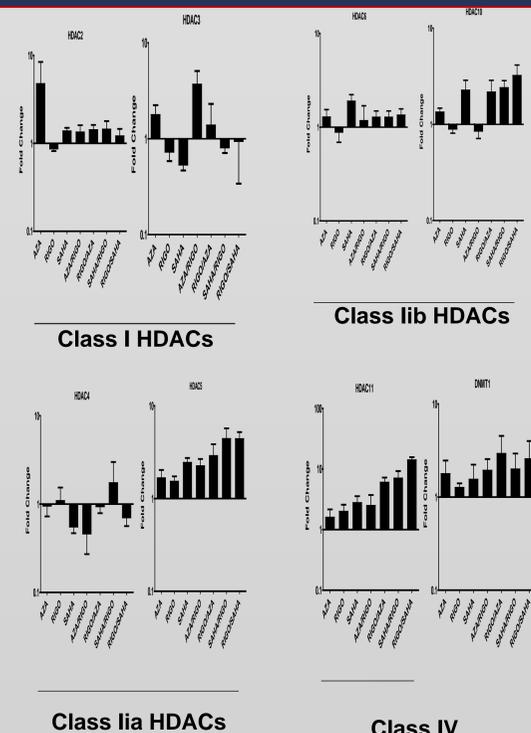
## BACKGROUND

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 (RIG) 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 RIG combined with AZA, in pts with MDS demonstrated a response rate of: 76% overall; 62% in pts following hypomethylating agent (HMA) failure and 85% in HMA naïve pts (Navada et al ASH 2016).

## MATERIALS & METHODS

We investigated the in vitro effects of RIG combined with AZA or vorinostat (VOR) on two cell lines: AML (BW90), MDS (MDS-L) and on pt bone marrow samples treated on the phase I/II Study, obtained prior to and after one cycle of AZA and RIG.

## RESULTS



Fold change in expression levels of HDACs gene and DNMT1 in MDS-L. (2A) Relative transcript levels of HDACs (Class I -HDAC, II and IV) and DNMT1 were calculated by SYBR Green Q-PCR. A relative mean normalized fold change in mRNA expression from each treatment conditions were normalized with the expression of these same genes in control cells. N=3-5

## RESULTS

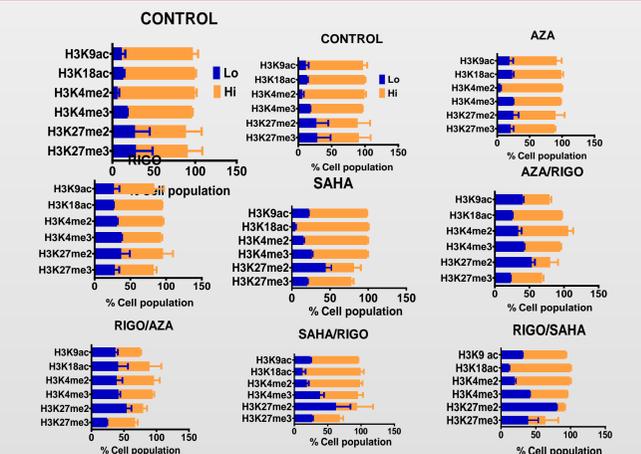
Treatment with RIG alone altered global histone post-translational modifications (PTMs) including methylation (H3K4me3, H3K4me2, H3K27me3, and H3K27me2) and acetylation (H3K9ac, & H3K18ac) levels associated with transcriptional activation or repression in both the cell lines and pt samples.

Q-PCR studies demonstrated that individual treatment of BW90 and MDS-L with RIG or combined with AZA or VOR in sequential treatment (AZA/RIG, RIG/AZA, VOR/RIG or RIG/VOR) altered DNA methyl transferases (DNMT1, 3a and 3b), the class I, II and IV histone deacetylases (HDACs), and chromatin remodeler (KDM2a, SET1, JMJD3 and LRWD1) transcript levels in a cell line specific context.

Sequential treatment of RIG with AZA or VOR demonstrated differential effects on the association of RNA polymerase II (Pol II) with active histone marks (H3K4me3 and H3K4me2) in both cell lines.

An overall decrease in association of Pol II/H3K4me2 was observed with the combinations (AZA/RIG, VOR/RIG or vice versa) in MDS-L and BW-90, 10-33% (ANOVA, p=0.0006), 9-20% (ANOVA, p=0.0004), respectively. Significant differences were observed in association of H3K4me3/Pol II in BW-90 cells (7-30%; ANOVA, p<0.0001). Similarly, in BM from a pt with MDS after 1 cycle of RIG and AZA treatment demonstrated a decrease in association of Pol II with H3K4me2 (67%) and H3K4me3 (28%).

In an MDS pt treated with RIG/AZA an expansion of primitive HPSCs expressing low levels of CD34 appeared with disappearance of a highly expressing CD34 subpopulation that co-existed prior to treatment. Expansion of CD34<sup>+</sup> cells led to ≥2 fold increase in pluripotent genes (SOX2, OCT4, NANOG and ZIC3) expression levels in the BM from MDS pts after RIG/AZA treatment and 1.7-34 fold increase in the presence of RIG or RIG/AZA or RIG/VOR in MDS-L. These findings indicate that expression of pluripotency genes is a consequence of epigenetic reprogramming that favors expansion of more primitive HSPCs in a pt.



**Effects of RIGO on global histone post-translational modifications in MDS-L cells.** 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 H3 lysine-9 and lysine-18 acetylation (H3K18ac and H3K9ac) following treatments with AZA, RIGO, AZA/RIGO or RIGO/AZA for 48hrs in MDS-L cells. On each row, the bar graph shows quantification of the percentage of MDS-L cells representing LO and HI distribution of various histone marks treated with CMAs either alone or in combination for 48 hrs. Two-way ANOVA P<0.0001

## CONCLUSIONS

RIG potentially functions as a chromatin modifying agent, in combination with AZA and may overcome HMA resistance through chromatin remodeling. RIG alone and in combinations also leads to epigenetic reprogramming of HSPC that may manifest in hematological improvements in the clinical setting.

## REFERENCES

Navada et al Combination of Oral Rigosertib and Injectable Azacitidine in Patients with Myelodysplastic Syndromes (MDS): Results from a Phase II Study. Blood, December 2016 ASH abstract