



## The 65th ASH Annual Meeting Abstracts

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## 636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

**Methylation Patterns of Fetal Hemoglobin Regulating Genes in High-Risk (HR) -MDS Patients Following Treatment with Azacytidine**

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**Background:** Current standard of care for HR-MDS patients, i.e. hypomethylating agent (HMAs) treatment with azacytidine (AZA) or decitabine (DAC), is associated with a 30-50% response rate, and many efforts have been made to identify predictive factors for response. Fetal hemoglobin (HbF) levels have been proposed as a prognostic index for DAC treatment (Stomper J. et al, 2019), but no study has examined its prognostic value in AZA treated patients, and the mechanism of  $\gamma$ -globin chain re-expression has not been investigated.

**Methods:** Bone marrow (BM) aspirates of 28 HR-MDS or CMML patients were collected at baseline and at the first evaluation of response, following 6 cycles of AZA treatment. Methylation of Cytosine followed by Guanosine (CpG) sites of the  $\gamma$ -globin gene promoter and CpG 326 of ZBTB7A/LRF, a previously characterized epigenetic repressor of HbF (Chondrou V. et al, 2022), were detected with pyrosequencing analysis in total genomic DNA, extracted from BM samples, pre- and post-treatment. Response to AZA was characterized as marrow response (mCR), when blasts were <5% and were reduced >50% from baseline, and as hematological response (HI-R) when there was at least one lineage improvement. HbF was measured with HPLC from the peripheral blood of 20 patients, at 5 time-points (before 0, 1, 2, 4 and 6-7 cycles of treatment), to test its expression pattern and potential prognostic significance. HbF values >1% were considered induced, as in previous studies (Stomper J. et al, 2019). For statistical analysis IBM SPSS 28.0.0.0 program was used. One patient with extremely high HbF before treatment (HbF<sub>0</sub>=13.4%) was excluded from the analysis, as well as the CpG site 3 of  $\gamma$ -globin gene promoter, which was 100% methylated pre- and post- AZA treatment.

**Results:** Median baseline HbF levels were 0.6% (range 0 - 2.6), and they were increased to 0.8% (range 0 - 1.8) and to 1.1% (range 0 - 3.5), at 2 and 6 months, post-AZA treatment, respectively. Difference in the repeated measurements of HbF was of borderline statistical significance ( $p=0.068$ ), specified between cycles 2 and 6 ( $p=0.038$ ). Correlation analysis showed a significant association of mCR with induced HbF after 6 cycles of treatment ( $p=0.011$ ,  $r_s=0.618$ ) and a strong positive correlation between elevation of HbF at cycle 2 and time to leukemia progression ( $p=0.019$ ,  $r_s=0.886$ ). Overall, median methylation of the  $\gamma$ -globin gene promoter was 73.4% and 72.8% pre- and post-AZA treatment, respectively ( $p=0.159$ ). However, patients exhibiting any kind of response showed reduced methylation at site 2 post- AZA, at the limit of statistical significance ( $p=0.095$  for mCR patients and  $p=0.066$  for HI-R), whereas site 4 was significantly hypermethylated among HI-R patients ( $p=0.042$ ). A negative correlation between methylation of the  $\gamma$ -globin gene promoter and gene expression was also identified ( $p=0.008$ ,  $r_s=-0.427$ ), particularly at the sites 2 and 5 ( $p_2=0.011$ ,  $p_5=0.004$ ).

Methylation analysis of CpG 326 of ZBTB7A/LRF showed significant hypermethylation at site 1 ( $p=0.044$ ) and reduction of methylation at site 22 ( $p=0.043$ ), following AZA treatment. When analysis was restricted among responders only, there was significant hypermethylation at site 1 ( $p_1=0.020$ ) and borderline significant hypermethylation at sites 5 and 11, among mCR patients ( $p_5=0.069$ ,  $p_{11}=0.079$ ). Conversely, sites 9 and 13 were significantly hypermethylated among non-responders ( $p_9=0.034$ ,  $p_{13}=0.047$ ).

With Spearman's analysis, a significant negative correlation between methylation fold change in site 13 and mCR was observed ( $p=0.002$ ,  $r_s=-0.555$ ). Although no correlation between HbF and CpG 326 methylation status pre- treatment was observed, after 6 cycles of AZA a borderline correlation was identified between HbF levels and fold change of mean methylation of CpG 326 ( $p=0.05$ ,  $r_s=0.482$ ). Moreover, induced HbF levels were related at the limit of statistical significance to increase in methylation at sites 1,3,5,11 and 22 ( $p_1=0.077$ ,  $p_3=0.052$ ,  $p_5=0.087$ ,  $p_{11}=0.061$ ,  $p_{22}=0.060$ ).

**Conclusions:** In this study, a relationship of  $\gamma$ -globin gene promoter methylation status and HbF levels was found among HR-MDS patients, treated with AZA. Moreover, hypermethylation in CpG326 of ZBTB7A/LRF after AZA treatment was associated with HbF levels, and different methylation patterns were observed between responders and non-responders. Further research is needed to clarify the role of LRF in MDS response to AZA.

**Disclosures Symeonidis:** *AbbVie:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Amgen:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *BMS:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Gilead:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Janssen:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Novartis:* Consultancy, Honoraria, Research Funding; *Pfizer:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau; *Roche:* Consultancy, Honoraria, Research Funding; *Takeda:* Consultancy, Honoraria, Research Funding, Speakers Bureau; *Sanofi:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding, Speakers Bureau.

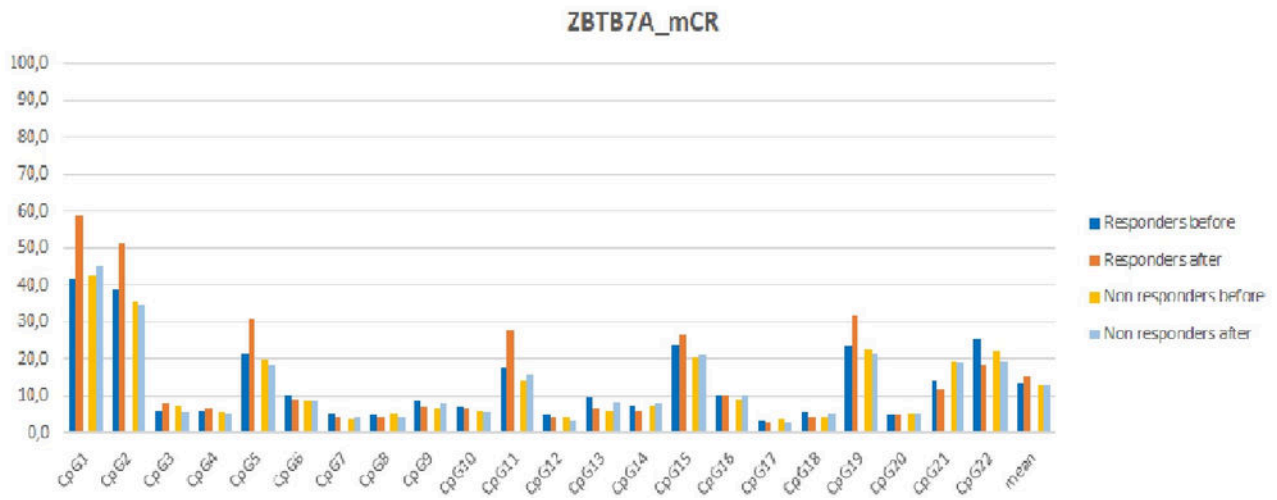
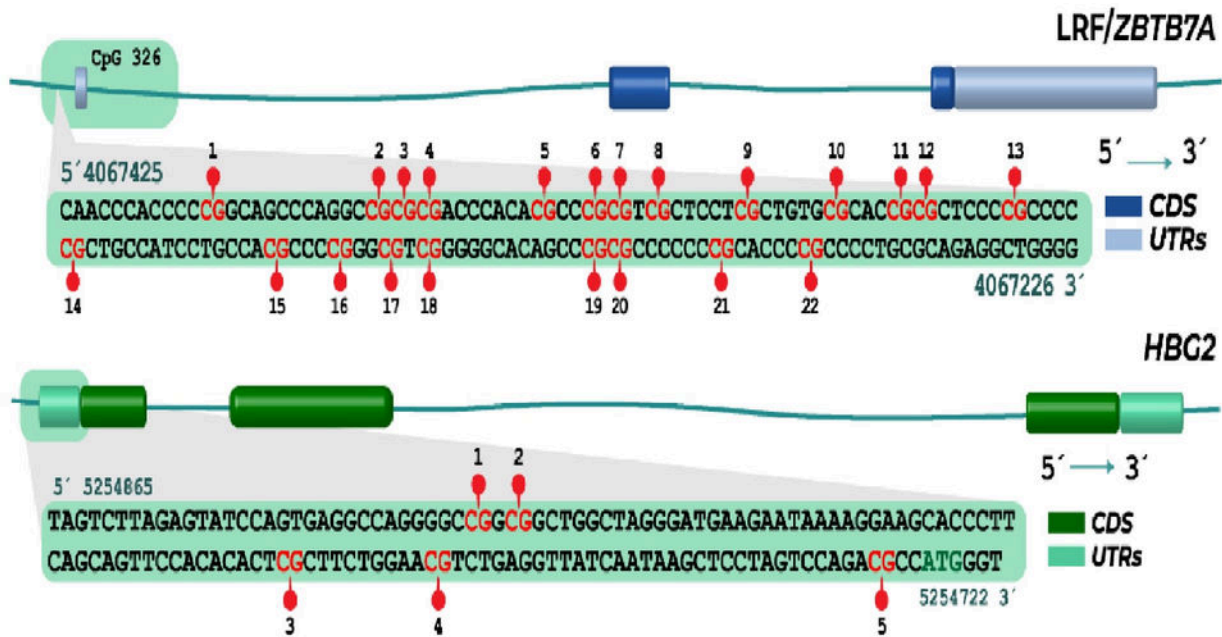


Figure 1

<https://doi.org/10.1182/blood-2023-187120>

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