XIX CONGRESSO NAZIONALE SIES2026
Firenze | 4-6 marzo 2026
Palazzo degli Affari

XIX Congress of the Italian Society of Experimental Hematology ABSTRACT N. CO40

Myelodysplastic Syndromes

DNA METHYLATION SIGNATURES ARE ASSOCIATED WITH CLINICAL RESPONSE TO AZACITIDINE IN HIGH-RISK MYELODYSPLASTIC SYNDROME PATIENTS

E. Lumia[1], A. G. Lema Fernandez[2], E. Fabiani[3|4], C. Nardelli[2], G. Falconi[3], E. Galossi[3], H. Hajrullaj[3], M. Crocioni[2], M. Quintini[2], A. Cristiano[1|5], P. Niscola[6], M.P. Martelli[2], R. La Tagliata[7], C. Mecucci[2], M.T. Voso[3]

[1]Department of Biomedicine and Prevention, PhD program in immunology, molecular medicine and applied biotechology, University of Tor Vergata; [2]Institute of Hematology and Center for Hemato-Oncology Research, University of Perugia and Santa Maria della Misericordia Hospital; [3]Department of Biomedicine and Prevention, University of Tor Vergata; [4]UniCamillus-Saint Camillus International University of Health Sciences; [5]Operative Research Unit of Laboratory, Fondazione Policlinico Universitario Campus Bio-Medico; [6]Division of Haematology, Sant' Eugenio Hospital; [7]Hematology, Belcolle Hospital, Italy

Introduction: High risk MDS (HR-MDS) outcome significantly improved after the introduction of hypomethylating treatment, especially 5'-azacitidine (AZA). Despite AZA is now the standard treatment for HR-MDS, only 30-50% of patients respond to treatment. The identification of response/resistance biomarkers is essential to prevent prolonged exposure and toxicities in unresponsive patients.

Methods: We used a comprehensive t-NGS, epigenetic (mERBBS), and transcriptomic (RNA-seq) approach to study 10 HR-MDS, prior and post 4-AZA cycles (5 responders, R, with complete remission; 5 non-responders, NR, with progressive disease). Healthy donor's (HD) BM samples were used as controls (n=5). 55 BM samples, from the GROM-L multicenter study (n=12) and Gimema MDS0205 study (n=43), were used as independent validation cohort.

Results: Age, gender, hematological and genetic features at diagnosis (T0) did not differ in the two subgroups. We identified a mean of 2 mutations (range=0–6, VAF>1%), in 8/10 patients at baseline, with TP53 (40%), DNMT3A (30%), TET2 (30%), ASXL1 (20%) and SETBP1 (20%) as the most frequently mutated genes. After AZA (T4), TP53 mutations became undetectable in 2/5 R patients (40%), while remained unchanged in NR. RNA-seq analysis at T0 (R vs HD, NR vs HD, R vs NR) and at T4 (R vs HD, NR vs HD, R vs NR) collectively showed different baseline transcriptional profiles in the two response-groups, particularly involving immune-related genes. Unsupervised analysis of DNA methylation separated the 2 subgroups, with one branch including all R (at T0 and T4) and HD, while the second all NR (at T0 and T4). mERRBS highlighted a DNA methylation gradient at diagnosis, in accordance with response. Indeed, NR was the most perturbed group, and the most hypermethylated. Within the hypermethylated promoters in NR at T0, BCL9L displayed the highest number of DMRs on its 5'-region. Pyrosequencing and qRT-PCR, were used to validate its hypermethylation and downregulation. Treatment with AZA induced a strong epigenetic shift only in R, with a reduction of DMRs (740 at T4 vs 2.732 at T0) and shift into a hypomethylated pattern (84.4%). By contrast, NR maintained at T4 a comparable number of DMRs (13.281 at T4 vs 13.231 at T0) and confirmed the hypermethylation seen at diagnosis (67.7%). Specifically, the methylation level at homeobox genes backed down to normal levels at T4 only in R. Within these DMRs, PRRX2 gene showed the stronger correlation with response. Hypermethylation in NR was confirmed in an independent validation cohort (n=5 R vs 5 NR, $p<0.005$).

Conclusions: Overall, our results identified a different transcriptional and epigenetic profile according to AZA response, including differential expression of immune-related genes and significant hypermethylation of NR at diagnosis, and a marked epigenetic shift after AZA in R. Of note, hypermethylation at BCL9L promoter and at the PRRX2 gene emerged as candidate predictive markers of AZA-response.