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Myeloproliferative disorders

AZACITIDINE REMODELS HEMATOPOIETIC STEM/PROGENITOR CELL FATE AND RESTORES ERYTHROID DIFFERENTIATION IN MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS WITH NEUTROPHILIA VIA REACTIVATION OF THE GATA SWITCH

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Myelodysplastic/myeloproliferative neoplasm with neutrophilia (MDS/MPN-N) is a rare and heterogeneous hematologic malignancy characterized by overlapping myelodysplastic and myeloproliferative features and recurrent mutations in *ASXL1*, *SETBP1*, and *ETNK1*. Current therapeutic options remain limited, with hydroxyurea providing only transient benefit and allogeneic transplantation being feasible in only few cases. The hypomethylating agent azacitidine (aza) has shown efficacy in high-risk myelodysplastic syndromes, but its molecular effects in MDS/MPN-N are poorly understood. This study aimed to characterize clinical responses and molecular mechanisms of aza in MDS/MPN-N through integrated clinical and single-cell transcriptome analyses.

Three patients with MDS/MPN-N received subcutaneous aza (75 mg/m^2, days 1–7, every 28 days) for up to nine cycles. Longitudinal hematologic, morphologic, and targeted NGS analyses were performed at baseline and after cycles 3, 6, and 9. Bone marrow CD34$^+$ cells collected at diagnosis and after three cycles underwent single-cell RNA sequencing to assess transcriptional and lineage changes. Complementary in vivo studies were conducted in a conditional *SETBP1-G870S* murine model treated with aza (5 mg/kg × 5 days).

Aza was well tolerated and achieved hematologic improvement in all patients, including normalization of leukocyte counts (A) and reduction in transfusion dependence (B). Bone marrow analyses revealed restored granulocytic maturation without disease acceleration. NGS monitoring showed heterogeneous clonal dynamics, indicating that clinical benefit was not strictly linked to clonal eradication.

Single-cell transcriptomics (C) demonstrated that aza profoundly remodels bone marrow architecture, reducing hematopoietic stem and early progenitor populations while expanding differentiating progenitors (D). Treated samples showed activation of cell cycle and differentiation programs (E) and repression of self-renewal drivers such as *MECOM*, *EYA1*, and *FLT3* (F). Gene ontology and GSEA analyses revealed enrichment of pathways related to chromatin remodeling, histone modification, and hematopoietic differentiation. In the erythroid compartment, aza promoted reactivation of GATA1-dependent transcriptional programs and downmodulation of *SPI1/PU.1* regulon activity (G), restoring the *GATA2*-to-*GATA1* switch (H-I) and enhancing heme biosynthesis and proliferation of erythroid precursors. Consistent with human data, *SETBP1*-driven leukemic mice treated with aza showed expansion of early erythroid progenitors.

Aza promotes extensive epigenetic and transcriptional remodeling in MDS/MPN-N, promoting differentiation and erythropoietic recovery while limiting aberrant stem cell self-renewal. Collectively, these data position aza as a rational therapeutic approach capable of modulating hematopoietic stem cell fate and achieving durable hematologic improvement in MDS/MPN-N patients who are not eligible for transplantation.

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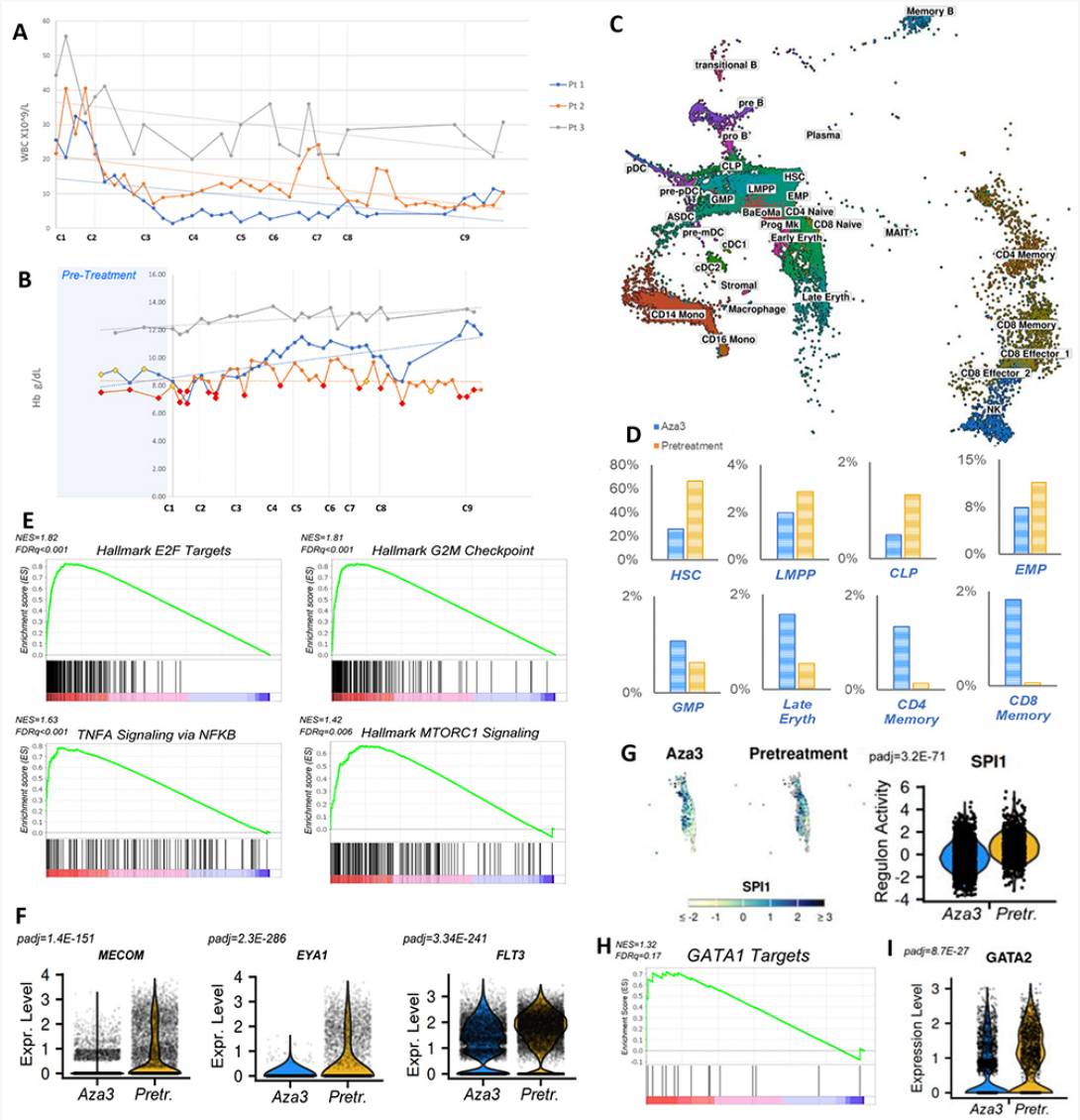

Figure 1. Clinical and molecular results of azacitidine treatment. Continuous blue, orange and gray lines represent longitudinal measurements of WBC counts (A) and Hb concentration (B) over azacytidine cycles for patient 1, 2 and 3, respectively. (C) UMAP plot relating to MDS/MPN-N patients before treatment and after the third azacytidine cycle and of healthy donors. Different colors highlight the main bone marrow cell populations. (D) Cell cycle analysis performed in the most immature bone marrow populations of MDS/MPN-N cases before treatment and after the third azacytidine cycle. (E) Gene Set Enrichment Analysis carried out on ranked genes identified in the HSC population in Azacytidine third cycle vs pretreatment analysis. (F) Violin plots showing the expression level of critical markers responsible for controlling the homeostasis of the hematopoietic stem cell compartment in MDS/MPN-N patients before treatment and after the third azacytidine cycle. Black dots highlight the expression level of individual cells. (G) Left panel: UMAP plot superimposed with the regulon activity level of SPI1 (PU.1) transcription factor in the early erythroid compartment of MDS/MPN-N patients before treatment and after the third azacytidine cycle. Right panel: Violin plots showing the regulon activity level of SPI1 (PU.1) transcription factor in the early erythroid compartment of MDS/MPN-N patients before treatment and after the third azacytidine cycle. (H) Gene Set Enrichment Analysis carried out on ranked genes identified in the early erythroid population in Azacytidine third cycle vs pretreatment analysis against GATA1 transcriptional targets. (I) Violin plots showing the expression level of GATA2 transcription factor in the early erythroid compartment of MDS/MPN-N patients before treatment and after the third azacytidine cycle.