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

XIX Congress of the Italian Society of Experimental Hematology ABSTRACT N. CO58

Myeloproliferative disorders

SINGLE-CELL MULTI-OMICS UNRAVELS MOLECULAR AND CELLULAR PROGRAMS UNDERLYING RUXOLITINIB RESPONSE IN MYELOFIBROSIS

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Introduction: Myelofibrosis (MF) is a clonal hematological disease originating by the sequential acquisition of somatic mutations in hematopoietic stem and progenitor cells (HSPC). Alongside so-called “driver mutations” triggering the constitutive activation of JAK-STAT pathway and inducing myeloproliferation, MF may present with additional mutations often affecting epigenetic regulators. The JAK-inhibitor Ruxolitinib (Ruxo) effectively relieves MF symptoms but rarely eradicates the malignant clone, with highly variable responses among patients. Understanding the molecular mechanisms underlying Ruxo heterogeneous efficacy is essential to improve therapeutic strategies.

Methods: To elucidate the clonal dynamics associated with Ruxo response, we conducted a longitudinal single-cell (SC) proteogenomic study on 12 MF patients (6 responders and 6 non-responders, as defined by clinical features) at diagnosis and after at least 6 months of Ruxo therapy. Cryopreserved peripheral blood mononuclear cells and $CD34^+$ HSPC from each time point were analyzed through Tapestri platform. Next, longitudinal samples from a Responder and a Non-responder patient form the same cohort were subjected to SC-RNA+protein analysis by means of 10X genomics platform.

Results: Ruxo responders showed a marked reduction in circulating $CD34^+$ cells and a decrease in JAK2V617F allele frequency in granulocytes. SC-genomic analysis revealed that the mutation acquisition order determines Ruxo sensitivity: patients in whom the driver mutation either occurred alone or preceded mutations in *TET2* or *ASXL1* generally responded to therapy, while those in whom epigenetic mutations arose first exhibited limited therapeutic benefit. Using SC-proteomics we identified 14 HSPC and differentiated cell clusters and differences in the clonal dynamics of $CD34^+$ and $CD34^-$ cells were observed. Non-responders displayed post-treatment expansion of highly mutated monocytes, suggesting myeloid lineage–driven resistance, whereas responders exhibited an increase in wild-type monocytes. Conversely, in $CD34^+$ cells from responders, we observed both a reduction in driver-homozygous cells and an expansion of heterozygous co-mutated clones despite Ruxo treatment. Notably, despite MF being a primarily myeloid disorder, a fraction of mutated T and B cells was also detected, particularly in non-responders. SC-RNA+protein profiling recapitulated the cellular heterogeneity observed in the SC-proteogenomic dataset, revealing persistent activation of JAK-mediated interferon-response signaling in non-responder monocytes upon treatment, while this pathway was suppressed in responders.

Conclusions: Our data demonstrate that the order of mutation acquisition impacts Ruxo response in MF patients. Ruxo primarily affects *JAK2*-only mutated clones. As a result, co-mutated clones may evade this treatment and outcompete other neoplastic cell populations, thus contributing to disease persistence.