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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

First-Hit SETBP1 Mutations Cause a Myeloproliferative Disorder with Bone Marrow Fibrosis

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Somatic *SETBP1* mutations are found in various myeloid disorders covering both myeloproliferative neoplasms (MPN) and myelodysplastic syndromes (MDS).

To characterize the early steps of SETBP1-mediated leukemogenesis, we generated a conditional mouse model expressing SETBP1 ^{G870S} mutant in the entire hematopoietic tissue through Cre-mediated recombination driven by the Vav1 promoter.
In all mice signs of a hematological disease appeared between 30 and 90 days: longitudinal analysis revealed accumulation of white blood cells (WBC) in virtually all heterozygous SETBP1 ^{G870S} mice, with a marked imbalance between the lymphoid and myeloid lineages in favor of the latter, and an increase in mature myeloid cells in absence of circulating blasts or non-segmented myeloid precursors. Kaplan-Meier analysis revealed a dramatic decrease in event-free survival in SETBP1 ^{G870S} mice.
BM histology showed overt myeloid hyperplasia with fibrosis and no evidence of dysplasia except for the megakaryocytic lineage. Exploration of visceral organs highlighted the presence of severe hepatosplenomegaly with massive infiltration by myeloid elements, disruption of normal tissue architecture, and signs of extramedullary hematopoiesis.
Single-cell RNA-sequencing (scRNA) on BM Lin $^{-}$ cells identified *Mecom*, *Setbp1* and *Hoxa9* among the top upregulated genes in SETBP1 ^{G870S} precursors.
Pseudotime analysis of scRNA data revealed the presence of 261 spatially autocorrelated genes. Of them 158 were also differentially expressed in SETBP1 ^{G870S} *vs* control mice. *Spi1*, encoding for the master regulator of hematopoietic differentiation PU.1, was significantly upregulated in SETBP1 ^{G870S} precursors. PU.1 promotes the maturation of bone marrow early precursors towards the granulocytic/monocytic lineages by directly impairing the transcription of *Gata2* and *Gata1*. In line with these data, *Gata2* and *Gata1* expression was profoundly suppressed in the early myeloid precursors of SETBP1 ^{G870S} mice, which associated with the down-modulation of markers of the erythroid lineage, such as the Carbonic Anhydrase 1, in the MEP differentiation branch.
Ourmouse model recapitulates many clinical features of primary myelofibrosis (PMF). As up to 10% of PMF are triple-negative for the classical *JAK2*, *CALR*, and *MPL* mutations (TN-PMF), we set out to assess *SETBP1* mutations in this context. We analyzed 36 TN-PMF patients by exome sequencing. In 29 we did not find any evidence of somatic mutations; in the remaining 7 (7/36; 19.4%) high VAF *SETBP1* degron mutations were identified. In two patients *SETBP1* was found as a single somatic variant, while in the others it coexisted with *ASXL1*, *NRAS TET2 SRSF2*, *RIT1 CBL* or *CSF3R* mutations. Notably, *SETBP1* mutations were the only shared alterations among all seven patients. A markedly reduced overall survival was observed for SETBP1 positive patients, with a median survival time of 24 months (median survival not reached at 60 months for SETBP1 negative patients).
To dissect the clonal architecture of SETBP1 positive TN-PMF at single-cell resolution, we applied single-cell targeted DNA sequencing on 3 SETBP1-mutated TN-PMF samples using the Tapestri technology, showing that, as opposite to MDS/MPN, in all TN-PMF cases *SETBP1* is a very early clonal event.
Therefore, our study suggests a clear partition of TN-PMF into two groups, the first one characterized by oncogenic, high VAF *SETBP1* mutations accompanied by other oncogenic variants and poor prognosis and the other one characterized by no evidence of an active clonal process or of driver oncogenic events and much lower aggressiveness.
SETBP1 positive TN-PMF disorders lie in a gray zone comprised between the MPN and MPN/MDS boundary. In MDS/MPN, *SETBP1* mutations are often found as mid or late events. In contrast, we show here that in TN-PMF *SETBP1* appears to be one of the earliest hits, therefore highlighting a potentially relevant biological difference occurring in the two subsets. In this context the presence of early *SETBP1* mutations seems to promote the occurrence of a myeloid disorder characterized by the triad: leukocytosis without differentiation block or dysplasia, BM fibrosis and progressive splenomegaly, hence recapitulating many clinical features of overt or prefibrotic/early PMF and resembling the SETBP1 mouse model.

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