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MEETING ABSTRACT

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A STAT5B–CD9 axis determines self-renewal in hematopoietic and leukemic stem cells

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Background: The JAK/STAT5 signaling pathway is essential in hematopoiesis and leukemogenesis. Activating STAT5 mutations in leukemia have been found exclusively in STAT5B, and despite considerable efforts no mutations in STAT5A have been identified. This seems to contradict the widely held belief that the two have essentially homologous functions. We now resolve the paradox by providing the first proof that STAT5A and STAT5B have different roles in hematopoietic stem cells (HSCs). Like HSCs, leukemic stem cells (LSCs) have the ability to self-renew and represent a major therapeutic challenge because of their high drug resistance, by stem-cell-specific properties including slow cell division, enhanced drug efflux or increased DNA repair. Therefore, it is important to identify novel mechanisms to target LSCs to enhance the effectiveness of curative approaches. The LSC-dependent diseases chronic myeloid leukemia (CML), acute myeloid leukemia (AML) or myeloproliferative neoplasm (MPN) examples include BCR/ABL^{p210}, FLT3-ITD or JAK2^{V617F}, where disease development requires STAT5 signaling. It remains enigmatic why STAT5B and not STAT5A is mutated, and whether the oncogenes activate both.

Methods: We used a broad range of *in vivo*, *ex vivo* and *in vitro* experiments to characterize HSCs and LSCs. We performed single-cell RNA-Seq to identify novel STAT5A and STAT5B target genes in HSCs, validated by ChIP-qPCR in murine hematopoietic progenitor cell lines. Finally, we translated our findings from various murine models to samples of human leukemia patients.

Results: We found a selective activation of STAT5B in HSCs and LSCs defining it as a key player in stem-cell quiescence and self-renewal, a function not shared by STAT5A. We generated single-cell RNA-Seq data to define a STAT5B-specific HSC signature and identified CD9 as a downstream target. Elevated CD9 levels are associated with a poor prognosis in AML patients. The elevated STAT5B/CD9 signaling in LSCs makes them sensitive to anti-CD9 antibodies, which induce differentiation and apoptosis.

Discussion: Our findings emphasize the importance of distinguishing between STAT5A and STAT5B. Our concept of selective STAT5B activation downstream of cytokine and oncogenic signaling paves the way for novel treatment approaches, here exemplified by CD9 blocking.

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Keywords: STAT5A – STAT5B – hematopoietic stem cells – leukemic stem cells – CD9

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