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A novel in vivo leukemia model demonstrates synergistic effects of mutations in *Asxl1* and *Csf3r*

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Abstract

Chronic Neutrophilic Leukemia (CNL) is a myeloproliferative neoplasm characterized by an overproduction of mature neutrophils. In over 90% of CNL cases, patients present with a mutation in *Colony Stimulating Factor 3 Receptor* (*CSF3R*), a receptor that plays a prominent role in proliferation and differentiation of neutrophils. When mutated, this receptor is aberrantly activated, leading to enhanced downstream signaling. A majority of these patients also harbor a mutation in *Additional Sex-Combs Like 1* (*ASXL1*), an epigenetic regulator of hematopoiesis, leading to disrupted hematologic gene regulation. The combination of those mutations confers poor prognosis. In this study, we used transgenic mouse models to determine the role of *ASXL1* mutations in the pathogenesis of *CSF3R*-mutant CNL.

Crossing a novel hematopoietic-specific *Csf3r*-mutant mouse model and an established *Asxl1* truncation mouse model, we created a four-genotype cohort to elucidate the effects of the single mutations as well as the double mutation. We validated our new model via PCR and Western Blot, and performed monthly complete blood counts, flow cytometry analyses of bone marrow, histology of tibias, colony-forming unit (CFU) assays, and blood smears. Our results show a premature age-dependent depletion of long-term hematopoietic stem cells (LT-HSCs) in *Csf3r* mutant mice, likely a result of LT-HSC exhaustion. Interestingly, when there is a co-mutation of *Asxl1*, this phenotype is reversed, and there is a LT-HSC expansion. Additionally, we found that hematopoiesis was skewed towards granulocytic lineages in *Csf3r* mutant mice, and this mutation triggers an aberrant C-X-C chemokine receptor type 4 (CXCR4) expression in neutrophils. CXCR4 aberrant expression was found to be exacerbated in the presence of the *Csf3r* and *Asxl1* co-mutation.

Our results suggest that the coexistence of *Asxl1* and *Csf3r* mutations results in a cumulative effect, promoting an overproduction of stem cells and neutrophil progenitors in the bone marrow.