EDITORIAL

Targeting leukemic stem cell dormancy in *NPM1c/FLT3-ITD*-driven acute myeloid leukemia

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In this issue of *Haematologica*, Boggio Merlo *et al*. present data on how nucleophosmin 1 (*NPM1*) gene mutations and internal tandem duplications (ITD) of the fms-related tyrosine kinase 3 (*FLT3*) gene co-operate in leukemic stem cells (LSC).[1] *NPM1* mutations generate an aberrant cytoplasmatic accumulation of NPM1c, and *FLT3-ITD* mutations result in a constitutive kinase activity. NPM1c/FLT3-ITD co-expression enabled LSC to replenish and preserve the dormant LSC pool, thereby preventing self-renewal exhaustion, while simultaneously driving selective pressure facilitating rapid selection and progression to acute myeloid leukemia (AML).

Leukemic stem cells are considered as disease-initiating cells at the apex of the AML hierarchy;[2] however, their phenotypic definition remains controversial given their significant plasticity. The capacity of LSC to acquire transient quiescence and dormancy states is thought to be a mechanism mediating resistance to anti-proliferative therapies. AML evolves following a series of founder and co-operating mutations, highlighting its heterogenous nature, and complex cytogenetic and molecular landscape.[3,4] A widely accepted model sees *NPM1* mutations as gatekeepers, suggesting that *NPM1*-mutant AML develops from pre-existing clonal hematopoiesis. *NPM1* and *FLT3* are frequently co-mutated in AML with a normal karyotype and co-occurring *NPM1*- and *FLT3*-ITD mutant alleles are associated with clonal dominance.[5] *NPM1* gene mutations have been associated with a favorable prognosis in the absence of concomitant *FLT3-ITD* in cytogenetically normal AML. In contrast, *FLT3-ITD* mutations drive high proliferation and are associated with an unfavorable prognosis due to high relapse rates and poor overall survival. AML patients with both mutations have a worse prognosis than those with only *NPM1* mutations, but a better prognosis than those with *FLT3-ITD* alone.[6] *NPM1*-mutant leukemias express a stem-like gene expression pattern that includes homeobox cluster A and B (HOXA/B) genes. HOX gene expression is directly dependent on *NPM1* mutants which act in a gain-of-function manner upstream of HOX to maintain the undifferentiated state of leukemic cells.[7] NPM1c supports the inappropriate expression of genes associated with HSC self-renewal throughout myeloid differentiation and it has been shown that the NPM1c-driven stem cell-associated program can be turned on at different stages of myeloid differentiation. The self-renewal properties induced by NPM1c in myeloid progenitors were sufficient to give rise to a pre-leukemic population that stably engrafted long-term, ultimately conferring enough self-renewal capacity to generate AML.[8] Activation of a humanized NPM1c knock-in allele in mouse hematopoietic stem cells caused Hox gene overexpression, enhanced self-renewal, and expanded myelopoiesis. *FLT3-ITD*-mutant hematopoietic stem cells (HSC) are hyper-proliferative and exhibit impaired self-renewal, with the mutant *FLT3-ITD* being both expressed and active in HSC.[9] However, there is also evidence that *FLT3-ITD* expression marks contaminating multi-potent progenitors (MPP), phenotypically overlapping with HSC. Murine models have shown that the frequently co-occurring *NPM1* and *FLT3-ITD* mutations are leukemogenic and induce AML in knock-in mice very rapidly,[10,11] whereas both individually led to late-onset AML or a myeloproliferative disorder after prolonged latency. Long-term HSC (LT-HSC) possess long-term reconstitution capacity, are serially transplantable, and steadily maintain the adult hematopoietic system. In their current study, Boggio Merlo *et al*. used murine models to confirm that NPM1c expression induces HSC proliferation and expansion by enhancing self-renewal. Concurrently, NPM1c-expressing LT-HSC maintained quiescence, preventing progressive exhaustion from continuous proliferative signals, such as those induced by FLT3-ITD. Given progressive exhaustion of the *FLT3-ITD* mutant HSC pool, the authors demonstrated that NPM1c co-expression rescued LT-HSC by preserving quiescence and HSC numbers, thereby preventing functional exhaustion. Interestingly, NPM1c enforced its transcriptional program and restored expression of HSC quiescence genes.

Hematopoietic stem cells either reside in quiescence or proliferate toward differentiation or self-renewal. Those HSC

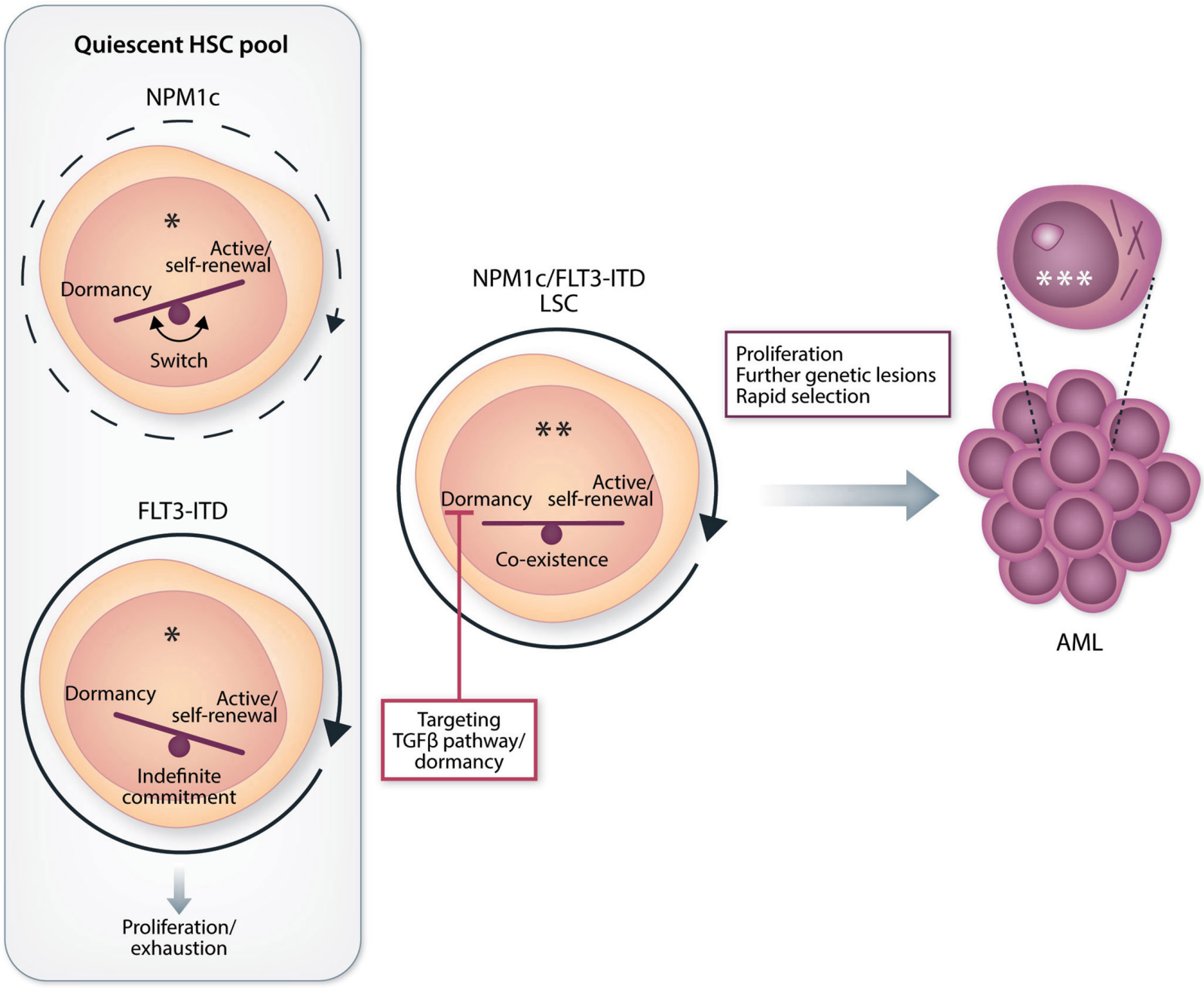

Figure 1. Schematic model illustrating the novel phenotypic state induced by NPM1c/FLT3-ITD co-expression, which fuels self-renewal and facilitates rapid selection of leukemic stem cells, as proposed by Boggio Merlo *et al.*

with the highest self-renewal capacity are maintained in a dormant state to preserve their activity but can be reversibly released to facilitate blood lineage repair.[12] Between these two functional states, dormant and active, HSC transition through many intermediate states.[12,13] In the current study, the authors used single-cell RNA sequencing and pseudotime trajectory inference to show that, while both dormant and active states were mutually exclusive in *NPM1* and *FLT3-ITD* single mutant mouse models, HSC in co-mutant mice adopted an intermediate state (Figure 1). This state was characterized by gene expression programs associated with both dormancy and proliferation, suggesting that NPM1c and FLT3-ITD co-operate to induce proliferation while preserving the self-renewal potential of LSC.

Finally, the authors focused on targeting the dormancy-related TGFβ pathway and assessed the repopulating potential of murine *NPM1/FLT3-ITD* mutant AML blasts previously challenged by *in vivo* pharmacological TGFβ1 inhibition. This experiment provided evidence that targeting dormancy-related pathways can reduce AML burden by impairing LSC self-renewal.

Collectively, the authors demonstrated in murine models that NPM1c reinforces quiescence by imposing its transcriptional program on *FLT3-ITD* mutant HSC, thereby rescuing their hyper-proliferative phenotype. This showed that both mutations co-operate to intervene in the balance between dormancy and activity in LSC to restore the quiescent stem cell pool. NPM1c stimulated the transition from dormant to active HSC and promoted re-entering of active HSC to dormancy through its enforced dormancy transcriptional program. This enables the replenishment of the dormant stem cell pool while preserving exhaustion of self-renewal capacity. In theory, this allows *NPM1/FLT3-ITD* mutant LSC to proliferate indefinitely and accumulate additional genetic alternations, facilitating their rapid selection (Figure 1). Overall, these findings illustrate how two of the most frequently co-occurring mutations in AML co-operate to drive LSC toward leukemia progression, while concomitantly preserving the LSC pool.

This also highlights the potential of single-cell sequencing technologies in capturing these dynamic cellular states. While further work is needed to establish the clinical significance of targeting dormancy through TGFβ1 inhibition in AML, this study underscores the critical need to integrate LSC-targeted strategies into AML therapies to eradicate leukemia-initiating cells alongside the bulk blast population.

Disclosures

No conflicts of interest to disclose.

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