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MYELOID NEOPLASIA

Comment on Rao et al, page 2139

"Interferon" with MPN hematopoietic stem cells

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In this issue of *Blood*, Rao et al reveal important insights into how hematopoietic stem cell (HSC) subpopulations contribute to myeloproliferative neoplasm (MPN) pathogenesis, and how these populations are perturbed in the setting of pegylated-interferon (pegIFN) therapy.[1]

The advent of pegylated forms of interferon (IFN) has provided renewed interest for use of this agent in the clinical setting. Recent studies demonstrate response rates on par with those of traditional cytoreductive therapy,[2,3] and lower rates of discontinuation have been observed as a result of an improved toxicity profile in comparison with their nonpegylated counterparts. Importantly, unlike other currently available MPN therapies (including JAK inhibitors),[4] IFN remains the only treatment in MPNs shown to reduce mutant clonal fraction and, in a minority of patients, induce molecular remissions,[5] suggesting that pegIFN can selectively impair clonal outgrowth at the level of the mutant MPN stem cell.

In recent years, sophisticated lineage-tracing techniques and single-cell approaches have revealed the striking heterogeneity of classically defined immunophenotypic stem cell populations[6] and called into question the traditional hierarchical model of human blood cell production. Megakaryocyte (Mk)-biased HSCs have emerged as an important branch point, with some data suggesting that Mk-biased HSCs can exist at the top of the hematopoietic hierarchy and retain self-renewal capability while also being able to differentiate directly into mature Mks.[7-9] This suggests that even at the primitive stem cell level, HSCs are already primed for cell-type–specific lineage output. It is hypothesized that the specific lineage-primed HSC population affected by a somatic MPN driver mutation might influence the phenotypic heterogeneity observed in MPNs. Improved understanding then of how MPN driver mutations, occurring in these individual HSC subpopulations, contribute to disease development, and, more importantly, how these cell populations can be influenced by disease-modifying therapies, will likely have critical clinical implications.

In their article, Rao et al are the first to evaluate Mk-biased HSCs in the MPN context. Using JAK2V617F mouse models as well as primary MPN patient samples, they evaluate the frequency and significance of CD41^{hi} (megakaryocytic biased) vs CD41^{lo} HSCs. Consistent with the expanded myeloid/megakaryocytic cell populations often associated with MPNs, the authors observe increased frequencies of Mk-biased CD41^{hi} HSC populations in JAK2V617F mice in comparison with wild-type controls. In clinical isolates, the frequency of CD41^{hi} HSCs is highest in polycythemia vera in comparison with other MPN subtypes and correlates with a JAK2V617F mutant allele burden, lending further support to the notion that quantitative dysregulation of JAK2 signaling itself can skew HSC subpopulations toward a particular lineage. Furthermore, the authors demonstrate that, although murine CD41^{hi} HSCs demonstrate enhanced Mk output in vitro, they lack MPN disease-initiating potential in transplantation assays. This suggests that CD41^{hi} HSCs, at least in MPN, represent a downstream, more committed population with reduced self-renewal capacity compared with CD41^{lo} HSCs. Consistent with this hypothesis, CD41^{hi} HSCs were more metabolically active and showed increased propensity to exit quiescence and enter the cell cycle than CD41^{lo} HSCs, an effect more pronounced in MPN mutant cells than those of wild-type HSCs. Critically, acute IFN pathway activation with polyinosinic:polycytidylic acid or more prolonged stimulation with pegIFN treatment resulted in a pronounced expansion of JAK2V617F CD41^{hi} HSCs with relative reduction in CD41^{lo} HSCs. Based on this, the authors suggest that CD41^{hi} Mk-biased HSCs in MPNs expand at the expense of primitive CD41^{lo} cells and, in turn, promote eventual exhaustion of MPN-sustaining CD41^{lo} HSCs and reduction in mutant clonal fraction over time with continued pegIFN therapy.

Although this important work provides a fascinating look at how Mk-biased HSC subsets expand in the setting of MPN and are skewed in response to IFN treatment, several intriguing questions remain. In the general context of hematopoiesis, it still remains unclear how these Mk-biased cell populations expand and/or differentiate in relation to other HSC subsets, specifically CD41^{lo} HSCs. Are preexisting CD41^{hi} cells already primed to expand in response to IFN, or are CD41^{lo} HSCs truly "converted" into CD41^{hi} HSCs with chronic IFN therapy? Furthermore, over what period of time are mutant stem cells "exhausted," and to what degree are MPN mutant stem cells preferentially sensitive to this effect? In addition, these data provide thought-provoking questions on the potential non–cell-autonomous effects of MPN mutant HSCs on wild-type CD41^{hi} HSC fractions and megakaryocytic output. Does the presence of a JAK2V617F mutant clone influence the frequency and cell output of its surrounding wild-type HSC counterparts? Finally, despite

showing that Mk-biased CD41^{hi} HSCs are expanded in MPNs, these cell populations appearing to lose their self-renewal potential suggests that still unknown factors, operating at the primitive stem cell level, are required for the phenotypic variability observed across MPNs.

As our understanding of the cellular heterogeneity of the HSC compartment improves, so too will our understanding of how MPN mutant stem cells survive to sustain disease. In turn, hopefully, we can design improved therapeutic strategies that better target MPN clones and those mutated cells at the earliest stages of the hematopoietic hierarchy. This work, taken together with recent clinical studies of IFNs in MPNs, provides a glimpse into MPN stem cell biology with the goal of informing additional therapeutic studies in MPNs and other stem cell–derived hematopoietic malignancies.

Conflict-of-interest disclosure: R.L.L. is on the supervisory board of Qiagen and is a scientific advisor to Loxo (until 2019), Imago, C4 Therapeutics, Mana, Auron, Ajax, Kurome, Mission Bio, Prelude, Scorpion, and Isoplexis, which each include an equity interest. He receives research support from and consulted for Celgene and Roche, he has received research support from Constellation, Roche, and Prelude Therapeutics, and he has consulted for Bridge Therapeutics, BMS, Lilly, Incyte, Novartis, and Janssen. He has received honoraria from Astra Zeneca, Constellation, Lilly, and Amgen for invited lectures and from Gilead for grant reviews. A.D. declares no competing financial interests. ■

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LYMPHOID NEOPLASIA

Comment on Patrussi et al, page 2182

IL-9 in CLL: sensing home and settling down!

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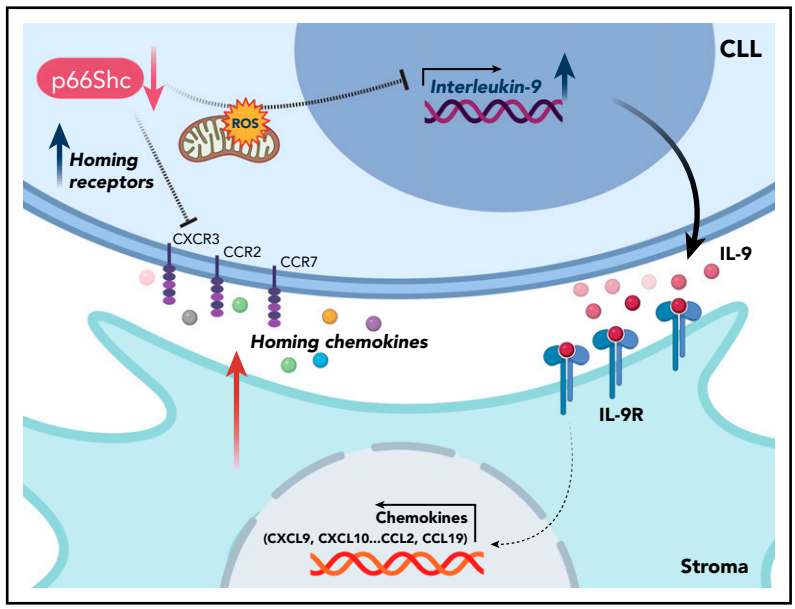
In this issue of *Blood*, Patrussi et al describe a complex, interleukin-9 (IL-9)–dependent, signaling pathway underlying the crosstalk between chronic lymphocytic leukemia (CLL) and stroma cells, highlighting the exquisite dependency of tumor cells on their microenvironment for survival and proliferation.[1]

The CLL microenvironment has ignited substantial scientific curiosity in the past decade and has ultimately revealed new vulnerabilities of tumor cells, which are now therapeutically exploited by B-cell receptor (BCR) inhibitors. Although this



Proposed model of the p66Shc-mediated IL-9 crosstalk between CLL and stroma cells. Low levels of p66Shc in CLL cells simultaneously enhance the expression of homing receptors and permit the transcription of IL-9 in an ROS-dependent manner. CLL-derived and secreted IL-9 binds to IL-9-receptors expressed on stroma cells and drives the expression of homing cytokines, further attracting CLL cells to their niche. Created with BioRender.com.