TP53 mutations cannot be excluded, this observation reflects the existence of multiple *TP53* mutated subclones in MM, which were not detected in the BM. Importantly, most of these patients did not have extramedullary disease, despite the fact that >40% of patients received at least 3 prior regimens.

When only BM results were examined in multivariate analysis, the presence of del(17p), *TP53*, and *KRAS* mutations was significantly associated with shorter progression-free survival (PFS). Indeed, although *KRAS* mutations have no prognostic impact on newly diagnosed myeloma,[8] they specifically negatively impact relapsed cases with *KRAS* mutations detectable in ctDNA (half the cases). Importantly, *TP53* mutations only detectable in ctDNA were also associated with poor prognosis. In addition, the number of detected mutations in ctDNA and plasma DNA concentration were also correlated with PFS. The authors had the opportunity to analyze paired samples before and after treatment; *TP53* and *KRAS* mutations were the most frequent emerging mutations in ctDNA. Finally, the authors proposed a prognostic scoring system able to successfully separate patients with relapsed/refractory disease into different risk subgroups, based solely on the number of mutations detected in ctDNA, plasma DNA concentration, and the number of lines of therapy.

This study brings a strong argument in favor of the value of ctDNA as a prognostic tool, detecting some aggressive subclones undetectable in the BM. However, if NGS was performed not at the bulk but at the single-cell level, the question arises whether these subclones would be identified.[9] In addition, the panel used here was not designed to capture copy number alterations, which have the strongest clinical relevance in MM. The poor prognostic impact of *KRAS* mutation only detected in ctDNA should be further explored: does it remain true at first relapse only? Does it only reflect tumor aggressiveness of the disease, or is there a real role for *KRAS* mutations in disseminating clonal heterogeneity? The fact that *KRAS* mutations are also associated with a shorter time to progression in smoldering myeloma may be in favor of the second option.[10]

The future of myeloma will undoubtedly lie in liquid biopsy. Nevertheless, as long as myeloma diagnosis requires BM analysis, the latter will remain the gold standard, and liquid biopsy a complementary tool.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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https://doi.org/10.1182/blood.2024024414

MYELOID NEOPLASIA

Comment on Laranjeira et al, page 2414

*Rela*ting NF-κB regulation to MPN pathogenesis

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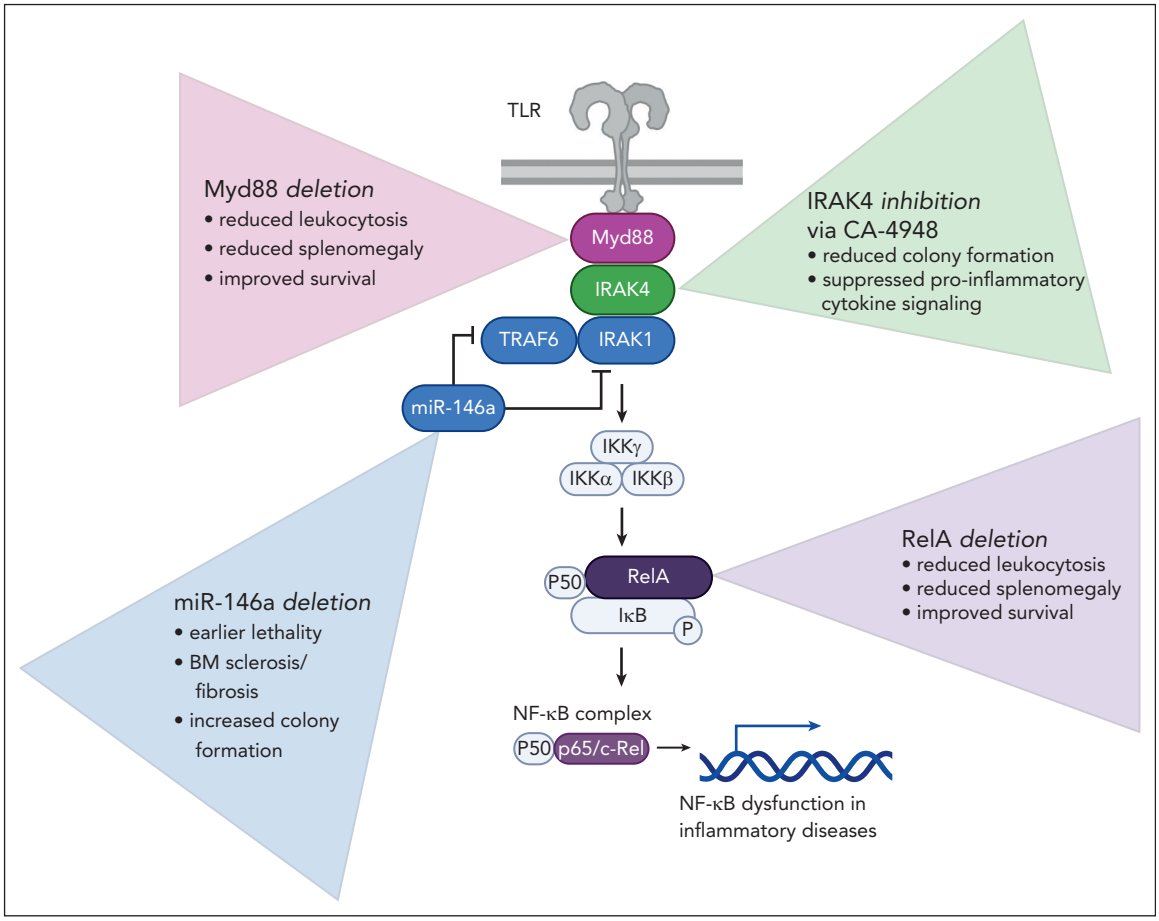
In this issue of *Blood*, Laranjeira et al[1] elucidate the role of NF-κB signaling in myeloproliferative neoplasm (MPN) pathogenesis by demonstrating how systematic disruption of key pathway components attenuate disease burden (see figure).

MPNs are myeloid malignancies driven by constitutively active JAK-STAT signaling and chronic inflammation. JAK inhibitors reduce inflammatory cytokines but not to the level of normal,[2] nor do they eliminate the underlying malignant clone. This persistence suggests that other pathways contribute to clonal survival. NF-κB is a family of inducible transcription factors that respond to diverse stimuli. Deranged NF-κB function is implicated in many inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis.[3]

Hyperactivation of NF-κB signaling is a characteristic feature of MPN, raising the question of how this intricate inflammatory pathway affects MPN pathogenesis.



Studies performed to dissect the role of NF-κB in MPN pathogenesis. Affects of loss of MyD88 and RelA were investigated in the context of Jak2^{V617F} knockin and MPL^{W515L} transduction transplantation mouse models. Disruption of miR-146a was investigated in primary patient samples and patient-derived xenografts. The role of IRAK4 in MPN was investigated with primary patient samples, patient-derived xenografts, and mass cytometry. BM, bone marrow; TLR, Toll-like receptor.

The NF-κB cascade is governed by numerous factors not yet fully evaluated in the context of MPN. The authors address this knowledge gap by using 2 syngeneic mouse MPN models, patient-derived xenografts (PDXs), and primary cells to investigate the dependency of NF-κB on MPN pathogenesis. With a series of comprehensive experiments systematically ablating NF-κB signaling pathway components, they provide novel evidence for the importance of Myd88 (myeloid differentiation factor 88) and miR-146a in MPN pathogenesis, as well as use RNA sequencing and mass cytometry to validate IRAK4 as a promising target for MPN.

First, to investigate the effect of *Rela* deletion on a polycythemia vera phenotype, they cross a ubiquitin C (UBC)-Cre-ERT2 mouse with 2 conditional models: *Jak2*^{V617F} knockin[4] and *Rela*$^{flox/flox}$-UBC-Cre-ERT2 (*Rela*$^{fl/fl}$).[5] This allows for the coincident expression of *JAK2*^{V617F} along with the deletion of *Rela* following tamoxifen exposure. The *Jak2-Rel* $^{fl/fl}$ transplanted mice cells have lower white blood cell counts, hematocrits, and spleen weights than mice transplanted with *Jak2*-UBC control cells. Next, the authors used an *MPL*^{W515L} transduction-transplantation model to explore how loss of RelA impacts a more aggressive MPN phenotype. Hematopoietic progenitor cells (c-kit^{+}) from *Rela*$^{fl/fl}$ or UBC-control mice were transduced with *MPL*^{W515L}-green fluorescent protein retrovirus, transplanted into lethally irradiated mice, and exposed to tamoxifen to induce loss of *Rela*. *Rela* ablation attenuates many of the *MPL*^{W515L}-driven disease hallmarks including leukocytosis, splenomegaly, and dysmegakaryopoiesis and extends life span in transplanted mice. PDXs further define the role of RelA in humanized MPN mouse models. Purified CD34^{+} cells from patients with myelofibrosis (MF) and normal bone marrow donors were transduced with a single guide RNA targeting RelA. RelA loss led to a reduction in neoplastic cells in peripheral blood and bone marrow, reduced spleen weights, and improved survival in mice transplanted with MF CD34^{+} cells. These results show clearly and concisely that *Rela* is a key mediator of pathogenesis in both mild and aggressive in vivo MPN settings.

The authors also asked how Myd88 and the microRNA miR-146a affect disease severity. Myd88, often studied in infectious models, is essential in interleukin-1 receptor (IL-1R) and Toll-like receptor signaling and has been shown to promote liver fibrosis and activate hematopoietic stem cells (HSCs) and the NLRP3 inflammasome in mice.[6] Hematopoietic progenitors from *Myd88*-null or wild-type mice were transduced with *MPL*^{W515L} and transplanted into recipient mice. The *Myd88*-null-*MPL*^{W515L} mice had attenuated leukocytosis and splenomegaly, extended survival, and reduced fibrosis as compared with the *MPL*^{W515L} mice.

In contrast to the activators RelA and Myd88, the microRNA miR-146a is a repressor of NF-kB, acting as a sort of suppressive "brake." Accordingly, ablation of miR-146a leads to excess myeloproliferation.[7] Here, Laranjeira et al demonstrate that disruption of *miR146a* enhances colony formation of both MPN and normal controls in vitro. In PDXs, disruption of *miR146a* reduces survival of mice injected with MPN CD34$^+$ cells but does not impact survival of mice injected with normal control CD34$^+$ cells.

To bring the experimentation to a more clinically relevant setting, they perform experiments with the specific IRAK4 inhibitor, CA-4948 (emavusertib), which is currently being investigated clinically in acute myeloid leukemia (AML). The authors support their reasoning for CA-4948's use in MPN with RNA sequencing demonstrating that MPN samples, most significantly those of MF and secondary AML, have upregulated *IRAK4* compared with normal. They further validate IRAK4 as a legitimate target in MPN, evidenced by dose-dependent inhibition of colony formation in MPN samples treated with CA-4948.

This work provides a new perspective on the dependency of MPN pathogenesis on multiple NF-κB targets and sets the groundwork for many follow-up questions. NF-κB is critical for HSCs, and a 2013 study by Stein and Baldwin found that *Rela*-deficient HSCs performed inferiorly to wild-type HSCs.[5] Here, the authors find only minimal effects of loss of *Rela* on HSC function, perhaps due to the use of an inducible system in the case of Laranjeira et al and constitutive *Rela* loss in the Stein and Baldwin article.[5] This raises the question of the source of these differences and the potential need to mitigate the mild but notable hematological effects of *Rela* and *miR-146a* perturbation in normal controls.

Another compelling question is the link between miR-146a, MPN, and autoimmunity. *miR-146a*-null mice have elevated inflammatory cytokines, myeloid proliferation, splenomegaly, and abnormal T cells, and they develop autoimmunity.[8] Given the coexistence of MPN and autoimmune disease,[9,10] this suggests dysregulation of the NF-κB pathway in MPN could be contributing to autoimmunity. Intriguingly, CA-4948 appears to be more effective against diseases with a preference for the long isoform IRAK4-L vs short IRAK4-S. What determines the ratio of short and long isoforms of IRAK4 in MPN? Investigating this partiality could assist in validating or maximizing the efficacy of IRAK inhibitors in MPN. Additionally, the reductions in colony formation from dual inhibition of JAK2 and IRAK4 warrant further in vivo experimentation on the antineoplastic benefits of simultaneously targeting these mechanisms.

Discovery of the *JAK2*, *MPL*, and *CALR* driver mutations changed the landscape of MPN research, but the unresolved need for a cure and more effective therapies leads us to wonder what other factors are at play. This study takes a significant step forward in establishing NF-κB as a bona fide target in MPN to not only address chronic inflammation but also ameliorate clonal expansion and fibrosis.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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https://doi.org/10.1182/blood.2024024451

THROMBOSIS AND HEMOSTASIS

Comment on Ryu et al, page 2425

Double-heterozygous FVL/PTGM: double the trouble

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In this issue of *Blood*, Ryu and colleagues[1] provide definitive data on the risk of venous thromboembolism (VTE) in individuals who are double heterozygous (DH) for factor V Leiden (FVL) and prothrombin gene mutation G20210A (PTGM). Although FVL and PTGM are the 2 most studied genetic risk factors for VTE, the risk of VTE for those with the DH genotype is less clear.

In 2001, a pooled analysis of case-control studies reported that the odds ratio (OR) for VTE in those with DH genotypes were 20.0 (95% confidence interval [CI]: 11.1-36.1), a significantly higher risk compared with heterozygous