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INSIGHTS

A knockout combination for MPN stem cells

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Myeloproliferative neoplasms (MPNs) are a group of blood cancers that are maintained by stem cell populations. In this issue of *JEM*, Dagher et al. (https://doi.org/10.1084/jem.20201268) combine arsenic and interferon α to deliver a knockout punch to MPN stem cells and provide new hope to cure patients with MPNs.

Myeloproliferative neoplasms (MPNs) are a group of clonal hematological disorders characterized by excessive production of mature myeloid cells including granulocytes, erythrocytes, and platelets. MPNs are driven by mutations arising in hematopoietic stem cells. Excluding chronic myeloid leukemia, which is pathognomonically associated with the BCR-ABL translocation, the classical MPNs include polycythemia vera, essential thrombocythemia, and primary myelofibrosis. These BCR-ABL–negative MPNs are primarily caused by driver mutations in JAK2, myeloproliferative leukemia virus (the thrombopoietin receptor), and Calreticulin. Mechanistically, these mutations all serve to constitutively activate JAK-STAT signaling, leading to the expansion of lineage committed progenitor cells and the corresponding disease phenotype (Vainchenker and Kralovics, 2017; Mullally et al., 2010).

Current therapies used in the management of MPNs include combinations of phlebotomy, aspirin, and cytoreductive therapy, most commonly hydroxyurea (Spivak, 2019). More recently, inhibitors of JAK2 signaling such as ruxolitinib have shown efficacy in controlling blood counts and treating symptoms, including splenomegaly, in patients (Harrison et al., 2012; Verstovsek et al., 2012). However, they are unable to eradicate the disease initiating MPN clone (Austin et al., 2020). Determining vulnerabilities that will allow the selective targeting of MPN-driver mutation–carrying stem cells is of key clinical importance, as it is likely to facilitate long-term disease control in patients and potentially also may reduce the incidence of transformation to secondary myelofibrosis or acute myeloid leukemia, a devastating complication of MPN associated with very poor long-term survival.

IFNα therapy has shown efficacy in the treatment of MPNs for many years; however, clinical use has been limited by the requirement for frequent injections and relatively high rates of toxicity. More recently, longer-acting pegylated versions of IFNα have become available with increased efficacy and reduced toxicity compared with the historical short-acting forms. In contrast to other agents, such as the Jak1/2 inhibitors, pegylated IFNα has been shown to induce reduction in the molecular burden of disease, in some cases leading to complete molecular remission, thought to represent a reduction in the frequency of MPN mutation–bearing cells (Kiladjian et al., 2008). These clinical responses are supported by data showing that IFNα is able to preferentially reduce the maintenance of $Jak2^{V617F}$ stem cells in murine models of disease (Austin et al., 2020; Mullally et al., 2013; Hasan et al., 2013). The mechanism behind this selective response to IFNα is not well understood, although it may be linked to increased DNA damage or failure to repair these processes (Austin et al., 2020). Molecular remissions appear to require prolonged treatment over a protracted time frame, and, therefore, it would be advantageous to determine combination strategies to further enhance this process in order to accelerate clinical responses. Importantly, Dagher et al. now demonstrate that arsenic trioxide (ATO) enhances the effect of IFNα in the treatment of MPN by depleting $Jak2^{V617F}$ mutant stem cell populations (Dagher et al., 2020).

Insights from Megan Bywater and Steven W. Lane.

ATO has a long therapeutic history related to its use in traditional Chinese medicine. In modern medicine, it has shown marked clinical efficacy in the treatment of de novo and all-trans retinoic acid–resistant acute promyelocytic leukemia (APL). de Thé and colleagues had previously shown that this effect was mediated by its ability to facilitate the degradation of the APL oncogenic fusion protein, PML-RARA (Zhu et al., 1997). Mechanistically, ATO can directly bind to PML and enhance nuclear body (NB) formation (Zhang et al., 2010). These PML-NBs facilitate the recruitment of proteins that both catalyze and interact with post-translational modifications. Of note, activation of p53 can occur via its direct recruitment to NBs through posttranslational

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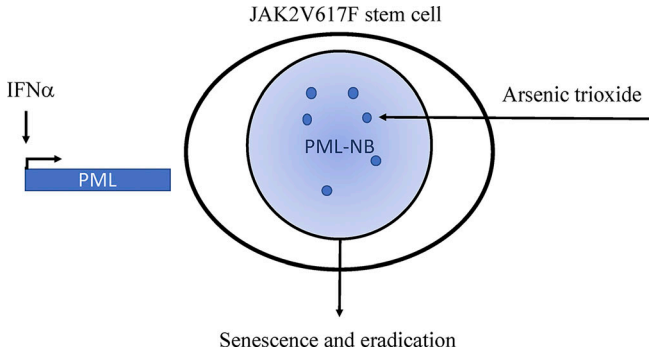

JAK2^{V617F} MPN stem cells are eradicated by ATO and IFNα. IFNα drives transcriptional expression of PML. ATO stabilizes PML-NBs. These processes lead to senescence and depletion of JAK2^{V617F} MPN stem cells.

modifications that alter its transcriptional activity and increased stability via the sequestration of Mdm2 (Bernardi et al., 2004). Increased PML-NB formation has also been shown to attenuate E2F transcriptional programs, most likely through direct recruitment and enhanced hypophosphorylation of Rb, resulting in growth arrest and senescence (Vernier et al., 2011). Consequently, PML-NB formation has been shown to have a tumor-suppressive role in a number of cancer models. Interestingly, PML has also been characterized as an IFN-stimulated gene (ISG; Stadler et al., 1995). These preliminary findings led Dagher et al. (2020) to ask whether increased PML-NB formation may contribute to the efficacy of IFNα therapy in the treatment of MPN and, furthermore, whether this effect could be enhanced by combining IFNα with ATO.

Dagher et al. (2020) were able to show that IFNα treatment increased the number of PML-NBs in primary human MPN CD34$^+$ cells and JAK2^{V617F} mutant human cell lines. Interestingly, JAK2 mutant CD34$^+$ cells isolated from MPN patients demonstrated an increased number of PML-NBs at baseline in comparison to wild type. As PML has been identified as an ISG, this finding is consistent with the JAK2^{V617F} mutation driving sensitization to IFN signaling, as inferred from the previous observation of higher basal transcriptional activation of Stat1 (Austin et al., 2020). Moreover, this also suggests that strategies to enhance PML-NB formation may be an effective way to selectively target Jak2 mutant stem cells. In support of this hypothesis, the combined administration of both ATO and IFNα was more effective at increasing PML-NB formation in comparison to either treatment alone and, importantly, was most effective in Jak2 mutant cells.

To functionally determine whether increased PML-NB formation was able to impact the long-term disease maintaining stem cell population, the authors next examined the effect of IFNα combination on the survival of JAK2^{V617F} mutant stem and progenitor cell populations. Here, combined IFNα+ATO was effective in reducing the colony formation capacity of both primary Jak2 mutant MPN patient samples and primary murine cells with the Jak2^{V617F} mutation knocked in to the endogenous locus. Impressively, the combination therapy proved effective in reducing not only MPN disease parameters in Jak2^{V617F} chimeric mice, including leukocyte counts, platelet counts, hematocrit, and splenomegaly, but most importantly in reducing the frequency of both mature myeloid cells and stem and progenitor cells expressing the Jak2^{V617F} mutation. Functionally, MPN stem cell populations were unable to transplant MPN into irradiated secondary recipients, an assay considered a gold standard of leukemia stem cell function. Additionally, after IFNα+ATO treatment was withdrawn, primary mice were monitored for the reemergence of disease. In >50% of the IFNα+ATO combination–treated mice, the MPN did not recur after treatment was stopped, demonstrating long-term treatment-free remission and potentially a cure of the MPN.

Next, to determine whether this selectivity was really dependent on PML-NB formation, the authors used shRNA targeting PML in Jak2 mutant CD34$^+$ MPN patient cells. Here, loss of PML reversed the effect of the IFNα+ATO combination to reduce colony formation in vitro. Next, to validate this in a genetically engineered murine model, murine Jak2^{V617F} mutant MPN stem cells were generated on either a wild-type or PML$^{-/-}$ background. In this context, the PML$^{-/-}$ mutant cells were preferentially selected during IFNα+ATO combination therapy, showing selective resistance to the effect of IFNα+ATO therapy. Finally, they provide preliminary evidence that enhanced PML-NB formation may lead to an eradication of MPN stem cells through the induction of senescence (see figure). This is shown through the accumulation of senescence-associated β-galactosidase and through increased transcription of senescence-associated genes.

In aggregate, this work provides a detailed mechanistic and functional validation of the effects of ATO in combination with IFNα in MPN stem cells. Specifically, this combination is able to deplete MPN stem cells, leading to reduced transplantation and even long-term treatment-free remission in disease control. Clinically, one would hope that these effects may manifest as molecular remissions and even cures. The findings in this paper are significant because they provide a very clear path to clinical translation. ATO is well established as a treatment for APL and is widely available, although limited by the need for parenteral therapy. Oral forms of ATO are also being developed. The toxicities of each agent are nonoverlapping, and one would suspect that this combination might be well tolerated clinically, in addition to the proposed beneficial effects. Future clinical studies to combine these two agents should be pursued in patients with MPN to carefully assess the clinical safety and efficacy of combining IFNα+ATO in patients with classical MPN with the long-term goal of achieving long-term treatment-free remissions, prevention of secondary transformation to leukemia, and potentially cure of the MPN.

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