through the phosphatidylinositol 3-kinase (PI3K)/ Akt/mTOR pathway-dependent increase in MDM2 translation, in cell lines and primary cells from MPN patients. Moreover, the cytokine-independent growth mediated by JAK2V617F in cell lines was inhibited using the inhibitor of MDM2/p53 interaction nutlin-3a, suggesting the important role of the MDM2/p53 axis in JAK2V617F-mediated proliferation.

Long-term IFNα treatments are usually required to obtain remission but are associated with frequent adverse effects such as fatigue, depression, weight loss, and nausea. Thus, using a drug combination might help lowering the doses or shortening the duration of treatment and appears as a good strategy for successful treatments. Based on previous data, Lu et al developed an original concept to target JAK2V617F malignant cells both by stimulating the p53 transcription via the IFNα and by inhibiting its JAK2V617F-mediated degradation using an MDM2 antagonist (see figure). In their work, they combined low doses of Peg-IFNα with a more potent nutlin derivative than nutlin-3a, called RG7112, which is already used in clinical trials while other second-generation derivatives are also currently tested. Interestingly, the authors make the proof of principle that this strategy is possible. In vitro, they observed an enhancement of progenitor cell death and a preferential targeting of JAK2V617F progenitor cells from PV or MF patients with the drug combination vs Peg-IFNα above, whereas RG7112 alone had no major effect at the dose used. These effects correlated with an increase in p53-target genes and subsequent apoptosis. More importantly, using a xenograft assay in immunodeficient mouse with pretreated CD34+ cells, they found a decreased engraftment after 4 to 7 months and a greater targeting of JAK2V617F cells with the combination compared with either RG7112 or Peg-IFNα alone. These results suggest that the drug combination leads to the preferential targeting of JAK2V617F stem cells. Even if these results are encouraging, it has to be noted that there is a great heterogeneity in the response of patients that could be due to other JAK2V617F-associated mutations (especially in MF patients) or to other mechanisms of resistance that are still not elucidated. Moreover, this study suggests that the IFNα and MDM2 antagonist act on stem cells through a cell intrinsic mechanism because in vitro the intervention from immunomodulation or from the microenvironment could be excluded. However, these findings have to be further confirmed in vivo because one major interest of IFNα treatment is to target quiescent HSCs by allowing their entry into the cell cycle. To do this, IFNα induces a relaxation of quiescent mechanisms that include a decrease in p53 gene expression that could be compensated by RG7112.

Altogether, this work further strengthens the role of the p53 axis as an important and druggable pathway in MPNs. This pathway seems to be crucial for the pathologic proliferation of JAK2V617F cells. Indeed, abnormalities in the p53 axis or p53 mutations have been frequently identified (>50%) in post-MPN secondary leukemia compared with other leukemia.9 Altogether, p53 abnormalities might be selected to drive the transformation of MPNs into leukemia. Therefore, the treatment of patients with nutlin derivatives in combination with IFNα might limit the cases of MPN transformation to leukemia. Finally, a similar strategy should also be considered for the treatment of chronic myeloid leukemia because BCR-ABL also up-regulates MDM2, and BCR-ABL-positive stem cells are triggered by IFNα. A main limitation of such an approach may be the thrombocytopenia, which is observed with each single drug treatment, more particularly with MDM2 inhibitors.

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

REFERENCES
The mDia1-deletion mice were created and analyzed. mDia1 deletion studies have demonstrated the potential consequences of this molecular pattern.

To mimic loss of mDia1 in del(5q) MDS and to understand the functions of formin protein family and plays an roles in hematopoietic stem/progenitor cell (HSPC) function, mDia1 is a RhoA GTPase effector that belongs to the formin family. mDia1 deletion in del(5q) MDS is a commonly deleted region in del(5q) MDS. mDia1-deletion and development of myeloid dysplasia in the BM.

mDia1-deletion mice also exhibit a striking age-dependent granulocytopenia, which is cell autonomous as reduced granulocytes also occur in wild-type mice transplanted with mDia1-deficient bone marrow (BM) cells (see figure). Consistent with a role in MDS, mDia1-deficient mice also have prominent myeloid dysplasia in the BM.

mDia1 is a RhoA GTPase effector that belongs to the formin protein family. mDia1 deletion in del(5q) MDS is a commonly deleted region in del(5q) MDS. mDia1-deletion mice also exhibit a striking age-dependent granulocytopenia, which is cell autonomous as reduced granulocytes also occur in wild-type mice transplanted with mDia1-deficient bone marrow (BM) cells (see figure). Consistent with a role in MDS, mDia1-deficient mice also have prominent myeloid dysplasia in the BM.

Analysis of myeloid markers on the surface of mDia1-deficient BM revealed high expression of CD14, a coreceptor for TLR4, leading to increased TLR4 signaling in granulocytes, granulocytopenia, and BM dysplasia.

Increased innate immune signaling has been previously reported in MDS. The first genetic evidence that increased innate immune signaling contributes to the pathogenesis of del(5q) MDS originated from observations involving loss of miR-146a, a microRNA within the deleted region on chromosome 5q. Knockdown or knockout of miR-146a in mouse HSPCs results in hematologic abnormalities resembling MDS, including impaired HSC function, peripheral blood cytopenia, and BM dysplasia, in part due to derepression of its target, tumor necrosis factor receptor–associated factor 6 (TRAF6). TRAF6 is a key signaling mediator downstream of TLR4. Given that mDia1 and miR-146a reside within 20 Mb of each other on chromosome 5q and are concomitantly deleted in del(5q) MDS, it will be imperative to create mice in which both genes are deleted. The primary hematopoietic defects associated with mDia1 deletion seem to be limited to granulocytes, whereas the defects following miR-146a deletion involve multiple hematopoietic cells, including long term HSC.

It is possible that the effects on long term HSC will not be exacerbated; however, the granulocytopenia might be significantly worsened in the double mDia1/miR-146a knockout mice. The molecular mechanisms resulting from chronic innate immune signaling are not entirely clear. Although TLR4/TRAF6 signaling activates nuclear factor–κB (NF–κB) under acute stimulatory conditions, it is still not known whether other pathways play a role following chronic activation. Notably, gene expression profiling of granulocytes from mDia1-deficient mice did not reveal increased expression of NF–κB target genes, except following LPS stimulation, suggesting that chronic innate immune activation may affect additional molecular and cellular components that contribute to del(5q) MDS.

The importance of the innate immune pathway in MDS was recently revealed by use of small-molecule inhibitors that target the TLR4 pathway. Interestingly, lenalidomide, the mainstay therapy for del(5q) MDS, was shown to extend the lifespan of mDia1-deficient mice and reduce the expression of CD14. Although the mechanism resulting in reduced CD14 expression is not known, some of the beneficial effects of lenalidomide in MDS may occur through “tuning down” innate immune signaling.

Innate immune signaling in HSPCs and mature immune cells is a tightly controlled process that involves many checks and balances. In contrast, inefficient regulation of the innate immune signaling pathway in HSPCs, such as by deletion of negative regulators (miR–146a) or overexpression of positive regulators (CD14, TRAF6, TLRs), contributes to defects associated with MDS. Deregulation of innate immune signaling could be a driving force in the pathogenesis of MDS. Additional investigation into the signaling pathways and creation of new mouse models will deepen our understanding and potentially uncover novel therapeutic options.
Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Koupenova et al, page 791

Platelets: crossroads of immunity and hemostasis

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In this issue of Blood, Koupenova and colleagues report that platelets express functional TOLL-like receptor 7 (TLR7) and contribute to host survival during viral infection. Through a series of experiments utilizing mice deficient for TLR7 together with adoptive transfer of wild-type platelets, Koupenova et al demonstrate that platelets specifically respond to viral analogs and intact virus, leading to platelet activation and binding to various leukocyte subsets. Perhaps most importantly, this platelet activation appears absolutely essential for host survival during infection with some viral pathogens such as encephalomyocarditis virus (EMCV).

It has long been known that severe viral infection often results in thrombocytopenia, although the specific mechanism(s) leading to this reduction in circulating platelets has remained elusive. Whereas some have suggested the loss of platelets is due to lysis and reduced production, others have provided evidence that platelets become sequestered within the microvasculature of organs such as the liver. Furthermore, it had been unknown if platelets were directly activated by the virus or if platelets were simply responding secondarily to the activation of other immune components. Previous studies demonstrated that platelets express functional TLRs and were able to directly detect and respond to bacterially derived pathogen associated molecular patterns (PAMPs) such as lipopolysaccharide and CpG DNA. Although these studies provided proof-of-concept that platelets were able to recognize pathogens, they failed to address questions surrounding the interaction between platelets and viruses.

In the current study, the authors demonstrate that in blood samples isolated from healthy human donors, platelets had measurable TLR7 protein expression. TLR7 is a pattern recognition receptor that specifically recognizes single-stranded RNA (ssRNA), a classic viral PAMP, contained within endosomes. Moreover, Koupenova et al were able to show, using either the TLR7 agonist Loxo or EMCV, that platelet TLR7 is functional, and ligand binding by this receptor results in platelet activation and degranulation. These studies represent a critical advancement in the field, as they clearly demonstrate that viruses are able to directly activate platelets to initiate a host response to infection. Interestingly, when platelet-derived mRNA from a large cohort of patients was examined, only ~60% of samples possessed transcripts for TLR7. This finding was particularly intriguing, as it suggests that platelets are equipped with their array of TLR7 molecules during development, possibly as part of the megakaryocyte, and although some platelets possess TLR7 mRNA, it is not required for the expression of functional TLR7 protein.

Although these experiments demonstrated that viruses are able to activate TLR7 on platelets, one must remember that TLR7 is present within endosomes and as such requires internalization of the virus to initiate signaling. Electron microscopy revealed both extensive binding of EMCV viral particles to the surface of the platelet (presumably due to the expression of sialic acid on the surface of the platelet) and a marked accumulation of viral particles within the platelet (possibly within primary lysosomes). Upon activation via TLR7, platelets undergo rapid and dramatic changes; using a series of well-devised experiments, Koupenova et al effectively elucidate the mechanisms underlying these changes (see figure). Rapid phosphorylation of Akt and p38-MAPK leads to fusion and release of platelet a-granules. This process results in expression of molecules such as CD40L and P-selectin on the surface of the platelet, allowing the platelet to adhere to leukocytes forming heterotypic aggregates (HAGs). During this activation, platelets undergo morphological changes, displaying pronounced membrane ruffling and extension of long, thin pseudopodia. These morphological changes allow the platelet to maintain intimate contact with the surface of the leukocyte. Over time, leukocytes, particularly granulocytes, are observed to internalize these adherent platelets (or possibly fragments derived from these platelets). Extending these findings from an in vitro model of platelet stimulation to a model of in vivo viral infection, the authors show that infection with EMCV leads to TLR7-mediated thrombocytopenia in mice and induces the generation of circulating HAGs and, importantly, that platelet TLR7 is essential for animal survival in this model.

Although platelets are classically considered to be central mediators of hemostasis, there is increasing evidence to show substantial overlap between thrombosis and immunity. Many aspects of coagulation appear to play a role in limiting pathogen
Errant innate immune signaling in del(5q) MDS

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