presence of \textit{BRAF}^{V600E}, clinical follow-up should be undertaken more vigorously because a reasonable proportion of patients harboring the mutation are destined to develop additional histiocytic disorders. Consequently, appropriate biomarkers for disease follow-up are urgently needed.

Mixed histiocytic disorders impose additional challenges in clinics because the response rates to traditional therapeutic regimens in these disorders are significantly lower compared with isolated disease. Also, there is no treatment of concomitant LCH and ECD, and mortality rate in these patients is relatively high. Because of the high incidence of \textit{BRAF}^{V600E} in MH, tyrosine kinase inhibitors ( vemurafenib) could be used as the gold-standard treatment. Furthermore, pan MEK inhibitors could be beneficial for patients demonstrating limited response to RAF inhibitors. Further studies are necessary to fully characterize mixed histiocytic disorders and determine the additional factors that could play a role in initiation and progression of these disorders. Eventually, multicenter studies are necessary to evaluate the efficacy of vemurafenib on overall survival of patients with MH.

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

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**Is JAK2\textsuperscript{V617F} finally off the hook?**

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In this issue of \textit{Blood}, Lamrani et al use various mouse models to demonstrate that the disease phenotype, rather than the JAK2\textsuperscript{V617F} mutation, is responsible for the increased risk of thrombosis and bleeding in mouse models of myeloproliferative neoplasms (MPNs).\textsuperscript{1}

MPNs are clonal somatic disorders characterized by the excessive production of mature myeloid cells such as erythrocytes and platelets and include polycythemia vera (PV; excessive erythrocyte production), essential thrombocythemia (ET; excessive platelet production), and primary myelofibrosis (MF; fibrosis of bone marrow). In 2005, 5 groups independently reported the presence of an acquired JAK2\textsuperscript{V617F} gain-of-function mutation in >95% of PV patients and >50% of ET patients.\textsuperscript{2} JAK2, a tyrosine kinase, is an essential player downstream of cytokine receptors such as the thrombopoietin (TPO) and erythropoietin (EPO) receptors, and a gain of function mutation contributes to the development of MPN. Paradoxically, patients have an increased risk of both thrombosis and bleeding, which are the main causes of mortality and morbidity in patients with MPN.\textsuperscript{2} Molecular changes in platelets, erythrocytes, leukocytes, and endothelial cells have all been suggested to contribute to the phenotype.\textsuperscript{2,5} However, the mechanism underlying MPN-associated hemostatic changes still remains largely elusive. As the risk of thrombosis is strongly associated with the JAK2\textsuperscript{V617F} gene load in both ET and PV patients, the question that has fascinated many scientists in recent years is whether the JAK2\textsuperscript{V617F} mutation is the direct underlying cause of thrombosis and/or bleeding in MPN patients.

Lamrani et al address this important question using hematopoietic-specific JAK2\textsuperscript{V617F} mouse models of PV/MF, which were generated by transplantation of bone marrow from constitutive (VarCre) and inducible (SCLCreER\textsubscript{T}) JAK2\textsuperscript{V617F} KI mice. What makes the inducible model unique is that it allows discrimination between the direct effect of JAK2\textsuperscript{V617F} expression and more long-term indirect disease-related effects. Using bone marrow transplants minimizes any contribution from JAK2\textsuperscript{V617F} expression in nonhematopoietic cells. In addition, the hematopoietic-specific JAK2\textsuperscript{V617F} mouse models of PV/MF were generated by transplantation of bone marrow from inducible (SCLCreER\textsubscript{T}) and constitutive (VarCre) JAK2\textsuperscript{V617F} knock-in mice. For the inducible mouse model, both the early (13 days after induction of JAK2\textsuperscript{V617F} expression) and late (60 days after induction of JAK2\textsuperscript{V617F} expression) phenotypes are indicated. The underlying cause of the observed phenotype, as suggested by Lamrani et al, is also included. √, phenotype observed.

<table>
<thead>
<tr>
<th>Phenotype of the JAK2\textsuperscript{V617F} KI mouse models used in Lamrani et al\textsuperscript{1}</th>
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<tbody>
<tr>
<td><strong>Inducible JAK2\textsuperscript{V617F} (SCLCreER\textsubscript{T})</strong></td>
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<tr>
<td><strong>Reduced in vitro thrombosis</strong></td>
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<td><strong>Reduced large vWF multimers</strong></td>
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<td><strong>Platelet hypocoagulability</strong></td>
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<td><strong>Prolonged bleeding</strong></td>
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<td><strong>Reduced platelet GPVI expression</strong></td>
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<td><strong>Vasodilation</strong></td>
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<td><strong>Clot lysis</strong></td>
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<td><strong>Rapid in vivo formation of unstable thrombi</strong></td>
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</table>

The hematopoietic-specific JAK2\textsuperscript{V617F} mouse models of PV/MF were generated by transplantation of bone marrow from inducible (SCLCreER\textsubscript{T}) and constitutive (VarCre) JAK2\textsuperscript{V617F} knock-in mice. For the inducible mouse model, both the early (13 days after induction of JAK2\textsuperscript{V617F} expression) and late (60 days after induction of JAK2\textsuperscript{V617F} expression) phenotypes are indicated. The underlying cause of the observed phenotype, as suggested by Lamrani et al, is also included. √, phenotype observed.
two non-JAK2V617F models were used to study the contribution of thrombocytosis/MF (TPOhigh retroviral RV model, in which marrow cells were retrovirally transduced with the murine TPO gene and grafted into irradiated wild-type recipient mice) and erythrocytosis (EPO-treated mice).

Analysis of these mouse models clearly revealed both direct and delayed effects of JAK2V617F on platelet functional responses, thrombosis, and bleeding (see table). Expression of both inducible and constitutive JAK2V617F resulted in the same hemostatic phenotype, providing additional confidence in these findings. JAK2V617F expression resulted in early effects such as platelet hyperactivity, decreased in vitro thrombosis, and increased bleeding time. The concurrent reduction in plasma high-molecular-weight von Willebrand factor (vWF) multimers, which support thrombosis, suggested that these impairments could be caused by vWF hydrolysis. A similar reduction in vWF multimers was recently observed in the Tie2-Cre/JAK2V617F mouse model of JAK2V617F.

Interestingly, a recent report by Hobbs et al., using a hematopoietic-specific JAK2V617F KI mouse model of ET (Mx1Cre/JAK2V617F), showed opposing results with increased platelet function in response to thrombin and collagen-related peptide and enhanced in vitro thrombosis. These differences may be explained by the MN phenotype of the 2 models, ET and PV/MF, respectively, or differences in gene load. Furthermore, it is unknown whether changes in vWF multimers occurred in the model used by Hobbs et al. Nonetheless, the decreased in vitro platelet function observed in the study of Lamrani et al is consistent with the in vitro platelet hyperactivity phenotype in MPN patients. A popular explanation for this is that platelets are hyperactive in MPN patients in vivo and become preactivated and exhausted. However, there was no evidence of platelet preactivation in the JAK2V617F models, arguing against this.

More delayed effects of JAK2V617F expression included a reduction in expression of the platelet collagen receptor GPVI and vasodilation of blood vessels. The reduction in GPVI levels was also observed in platelets from TPOnh high retroviral mice, which have symptoms of MF, without the hemostatic changes observed in the JAK2V617F mice. Reduced GPVI expression is therefore unlikely to be involved in the observed reduction in in vitro thrombotic response because (1) TPOnh mice do not show these hemostatic changes, and (2) it occurs later (60 days). Vasodilation was observed in both JAK2V617F and the EPO-treated mice and could therefore be the result of increased blood viscosity due to the higher erythrocyte count and subsequent vessel adaptation.

One of the more interesting delayed effects of JAK2V617F expression was the accelerated formation of occlusive thrombi in a FeCl3 in vivo thrombosis model. These results are consistent with the increased risk of thrombosis in MPN patients. The thrombi, however, were less stable, with increased embolization and clot lysis being observed. Interestingly, the Tie2-Cre/JAK2V617F model also showed increased thrombus instability and embolization. Although thrombin generation was not determined by Lamrani et al, thromboelastometry showed that in vitro tissue factor-induced coagulation was delayed in JAK2V617F platelets, which may contribute to thrombus instability.

Is the JAK2V617F mutation directly responsible for the increased thrombotic risk of MPN patients? The data in the study of Lamran et al demonstrate that it is more likely related to the progression of MPN disease than a direct result of JAK2V617F mutation. Although the study cannot completely rule out a direct role of JAK2V617F in the early changes in platelet function, recent work by Etheridge et al. showed that platelet function was unaltered in mice expressing JAK2V617F (PF4-CRE/JAK2V617F) in megakaryocytes only. This is consistent with patient studies, where impaired in vitro platelet function in ET patients was unrelated to JAK2V617F status. Thus, although JAK2V617F in platelets may not directly affect platelet function and the prothrombotic phenotype, the possibility that it contributes at a later stage of the disease cannot be completely ruled out. These findings are also interesting in the light of recent publications describing a role for erythrocytes, leukocytes, and endothelial cells in hemostatic changes in MPN mouse models and the potential impact of JAK2V617F on the prothrombotic potential of erythrocytes and endothelial cells. This leaves open the question as to whether JAK2V617F can contribute directly to the prothrombotic phenotype. This, plus a more detailed understanding of the cellular mechanisms that underlie the hemostatic disorders in the complex spectrum of MPN, will form important new directions for the future in this work, building on the important contribution made here.

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

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A step forward back to (induced) fetal

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