recurrent somatic insertions and/or deletions (indels), creating de novo transcription factor binding sites for MYB, ETS1, or RUNX1 (see figure panel B). Given that these factors are all part of an oncogenic protein complex11 that also includes TAL1 and LMO2 itself, it is tempting to speculate that these newly identified noncoding mutations will ultimately trigger the formation of an autoregulatory loop that will further boost oncogenic transcription during malignant T-cell transformation.11 Only the intronic LMO2 mutations that created a de novo MYB binding motif were functionally validated. Nevertheless, the fact that specific mutations also created ETS1 or RUNX1 recognition sites in close proximity of a preexisting MYB motif suggests that cooperative but interchangeable activity of different transcription factors could contribute to oncogenic LMO2 activation. This seems to be different as compared with the MYB centered mechanism-of-action that was suggested for the previously identified TAL1 enhancer mutations.7 Interestingly, another study recently reported a very similar mechanism of LMO2 oncogene activation in T-ALL.12 However, in that case, the somatically acquired noncoding mutations, which also created a de novo MYB binding motif, were located further upstream of the LMO2 TSS and created a somatically acquired enhancer that triggered aberrant monoallelic expression of LMO23,7 (see figure panel B).

In a complementary study, also presented in this issue, Hu et al performed whole genome sequencing analysis on a cohort of 31 pediatric T-ALLs and confirmed the presence of the previously identified TAL1 noncoding alterations mentioned earlier. However and most notably, their unbiased approach also enabled the identification of a recurrent point mutation in a noncoding area upstream of the TSS of the LMO1 proto-oncogene (see figure panel C). Similar to what has been described for TAL18–10 and LMO2, this somatically acquired single-nucleotide variant created a de novo MYB recognition site, triggering the formation of an aberrant transcriptional enhancer complex that drives high levels of monoallelic LMO1 expression (see figure panel C). Interestingly, this oncogenic enhancer mutation at the LMO1 locus might occur as part of an APOBEC-induced mutational signature, as recently described by Li et al.13

In conclusion, Rahman et al and Hu et al confirm that somatic alterations that drive aberrant activation of neomorphic promoters or enhancers14 can be critically involved in malignant T-cell transformation. These studies further complement our molecular genetic understanding of human T-ALL and reinforce the critical importance of the oncogenic TAL1-LMO complex in T-ALL disease biology. In addition, it is tempting to speculate that other oncogenes involved in the biology of this disease, such as NOTCH1, MYC, TLXI, TLX3, HOX4, NKX2.1, or NKX2.2.5 could also be activated through similar mechanisms. In contrast, one could also imagine a scenario in which noncoding alterations disrupt certain transcription factor binding motifs, thereby causing inactivation of an enhancer in the proximity of a certain tumor suppressor gene. Altogether, these studies provide a strong rationale to further explore the landscape of somatic noncoding alterations in human cancer.

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

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DOI 10.1182/blood-2017-04-773242
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Comment on Guglielmelli et al, page 3227

Pre-PMF emerging as important subgroup of MPN

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In this issue of Blood, Guglielmelli et al highlight the importance of distinguishing prefibrotic/early primary myelofibrosis (pre-PMF) from overt primary myelofibrosis (overt-PMF) as a separate prodromal subgroup of myeloproliferative neoplasm (MPN).1 Before the World Health Organization (WHO) 2016 classification, pre-PMF was neither generally accepted in the scientific community as a distinct subgroup of MPN nor aligned with overt-PMF.2 In this study, the authors provide evidence that pre-PMF represents a prodromal stage of overt-PMF, and that it is clearly different from essential thrombocythemia (ET). With respect to clinical, hematologic, and molecular...
phenotypes, pre-PMF is associated with better risk factors that result in prolonged overall survival, as well as leukemia-free and fibrosis-free survival, when compared with overt-PMF. However, as has been reported in earlier studies, pre-PMF showed a significantly shorter overall survival than ET. Patients with pre-PMF are younger compared with patients with overt-PMF. Patients with overt-PMF have greater hematologic abnormalities (anemia, leukopenia, thrombocytopenia, higher blast counts), are more symptomatic, have larger spleens, and have a higher frequency of high molecular risk mutation profiles. Interestingly, the cohort of patients with pre-PMF had a predominance of female patients, which suggests the possibility that male patients progress to overt-PMF faster as the sex distribution is balanced in the later cohort.

Since the late 1990s, a number of clinicopathological studies including follow-up examinations with repeated bone marrow (BM) biopsy evaluations have validated the existence of a precursor stage of overt-PMF (reviewed by Barosi, this finding was not recognized in classification systems). The results of the present paper are very supportive of pre-PMF being its own subset among MPN, but should stimulate further clinical research and will, certainly, change routine procedures in clinical practice. First of all, this investigation confirms that it is mandatory to perform a BM biopsy in all patients newly diagnosed with MPN to estimate survival and optimize treatment decisions. Second, the present investigation demonstrates that spleen size and grade of BM fibrosis, as well as lactate dehydrogenase level, hitherto underreported, may display the severity of the disease. Third, when the International Prognostic Scoring System (IPSS) is applied in pre-PMF, the striking difference in clinical phenotypes between pre-PMF and overt-PMF influences the predictive value of this score with a superimposable median survival of more than 10 years for patients scored as intermediate 1 or intermediate 2. The IPSS, important for decision making for allogeneic stem cell transplantation in overt-PM, may therefore not be an adequate tool for such decisions in pre-PMF. Finally, and most importantly, the authors show that a high molecular risk profile is associated with a shorter survival not only in overt-PMF but also, when present, in pre-PMF. This is a new finding and will certainly change clinical procedures.

This study in a multicenter, real-life scenario demonstrated that the WHO 2016 classification is reproducible, with the caveat that the assignment to the different subgroups of MPN was not centrally reviewed by experienced hematopathologists. This study should stimulate similar analyses with descriptions of clinical and molecular differences between ET and pre-PMF, as this distinction is becoming more and more important for treatment decision making with the development of new treatment options for MPN. Finally, the prevalence of an MPN subgroup of pre-PMF comprises 15% to 25% of all patients with MPN; however, the exact numbers can only be determined by evaluations in centers with a diagnostic mandatory BM biopsy examination at entry of the patients (see figure). The overall survival of this patient group is comparable to that of patients with PV. With this new landmark analysis, it is clear that pre-PMF cannot be ignored as a separate subgroup of MPN. It requires management strategies that are different to those of ET and of overt-PMF. As suggested by the authors, prospective clinicopathological studies should be launched, including centralized evaluation of diagnostic BM biopsy slides, not only to establish the prevalence of pre-PMF and to confirm morphological features but also to
further confirm the reproducibility of the WHO-defined criteria of pre-PMF.

Conflict-of-interest disclosure: H.G. receives research funding from Novartis and AOP Orphan, as well as speakers honoraria from Novartis, AOP Orphan, and Shire.

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DOI 10.1182/blood-2017-04-777805
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THROMBOSIS AND HEMOSTASIS

Comment on Mukhopadhyay et al, page 3245

From tumor suppressor to thrombus resolver

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The gene p53 was originally identified in 1979 as a tumor suppressor in a multitude of human cancers (eg, colorectal, lung, and breast), where it functions as a suppressor of cancer proliferation by means of the induction of cell cycle arrest and programmed apoptosis in response to a variety of cellular stress signals. Loss of p53 or the expression of p53 gain-of-function mutations can enhance the metastatic potential of tumor cells. However, p53 has subsequently been found to be involved in a variety of fundamental gene processes, including gene transcription, the cell cycle, the abovementioned programmed cell death, DNA replication, and DNA repair. Now, for example, p53 is known to regulate and mediate vascular remodeling and wound healing, with effects on adhesion molecules and, relevant to fibrinolysis, the expression of plasminogen activator inhibitor (PAI-1).

In this regard, in this issue of Blood, the article by Mukhopadhyay et al reports the novel finding that endogenous p53 activity in cells of myeloid lineage plays a significant role in macrophage polarization towards a tissue remodeling phenotype and thereby resolution of venous thrombus formed using a mouse vena cava ligation model. By resolution is meant inflammatory-mediated vascular remodeling that directly involves the thrombus. In particular, intrathrombus collagen content by Picrosirius Red staining was reduced by 30% to 35% in wild-type mice, as compared with Mip53+/− mice, that lack myeloid p53, at day 12 (see figure).

The authors further report that the genetic deficiency of p53 (Tp53−/− mice) or pharmacologic inhibition by daily intraperitoneal pifithrin-α, a small-molecule inhibitor of p53 function, leads to a significant reduction in the inflammatory state associated with major thrombus formation, the inflammatory state itself being a requirement for thrombus resolution. Conversely, pharmacologic augmentation of p53 activity using subcutaneous infusion of the anti-malarial quinacrine, a p53 agonist, accelerates resolution of the “well-established” thrombus present 3 days following ligation. Treatment with quinacrine, a known stabilizer of p53 and inducer of both p53-dependent and p53-independent cell death, results in a 40% reduction in thrombus weight by day 12, as shown in Figure 6 in the article by Mukhopadhyay et al. The quinacrine effect, moreover, is specific to myeloid p53, since treatment of lysM-Cre/p53 mice, deficient in myeloid p53, has no effect on venous thrombus resolution.

Quinacrine, taken up into platelet-dense bodies and endothelial cells as well as leukocytes, may have other p53 cellular actions not presently described, if not ones that bear on thrombus resolution. Further, resolution of thrombus fibrin is not addressed explicitly, and the full implications of the discovery of the above role for p53 for venous thrombosis are not yet known. For example, might certain p53 mutations contribute to hypercoagulability? Can some measure of macrophage polarization become a biomarker for clot resolution? Since blood flow directs convective diffusion of platelets and procoagulant proteins and has shear stress–mediated effects on cellular function, as well as receptor–ligand bond lifetimes, what might be the effect of blood flow on p53 function?

Nonetheless, the findings of Mukhopadhyay et al suggest a novel pathway to which treatment of venous thrombosis may conceivably be extended in the future. Beyond quinacrine and other acridine derivatives, perhaps other agonists will now be found that, alone or in combination with newer anticoagulants and/or systemic or catheter-directed exogenous thrombolitics, will have even greater and/or more rapid benefits in thrombus resolution while retaining an acceptable safety profile. Such therapies are needed if we are to avoid early and irreversible damage to the venous valves, which underlies a large part of venous...
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Heinz Gisslinger