transition of LFA-1 integrin into a high-affinity activated state. It should be noted that Rac1 may also be involved in other signaling pathways, which may induce LFA-1 activation independently from Plcβ2/3. Further studies are needed to characterize the components of these pathways and to define the relative contribution of the possible complementary pathway to LFA-1 activation.

To demonstrate the in vivo relevance of their findings, Block et al used mixed chimeric mice in which Gβ subunit isoform–deficient and control neutrophils can be compared with each other in the same animal. The authors demonstrated that all Gβ subunit isoforms are critical for neutrophil recruitment to the lung in a lipopolysaccharide-induced lung injury model, indicating the importance of the pathway in vivo. Furthermore, the authors demonstrated that Plcβ2, Plcβ3, and Rac1 deficiency reduced the recruitment of neutrophils to the inflamed lung.

The excellent study by Block et al raises several additional questions. Currently it is not clear why the Gβ subunit isoforms show a nonredundant role in chemokine-dependent integrin activation. It is possible that the Gβ subunit isoforms act in the same macromolecular complex. Alternatively, each isoform may be involved in specialized functions, all of which are indispensable for integrin activation. Further investigations are needed to distinguish between these scenarios. The question of whether the activation of other integrins depends on the same signaling machinery should also be addressed. The unexpected role of Rac1 in proximal signaling suggests important questions about the function of the small GTP-binding protein. Revealing novel molecular mechanisms of chemokine-induced inside-out signaling has great importance because modulating integrin activation is a perfect way to regulate leukocyte recruitment and activation at the inflammation site; therefore, components of the integrin inside-out signaling pathways might be important novel therapeutic targets.

In conclusion, Block et al have provided important insights about the role of G protein Gβγ subunits in chemokine-induced signaling through Rac1 and Plcβ2/3 leading to LFA-1 activation in neutrophils.

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● ● ● MYELOID NEOPLASIA

Comment on Milosevic Feenstra et al, page 325, and Cabagnols et al, page 333

Closing the gap: genetic landscape of MPN

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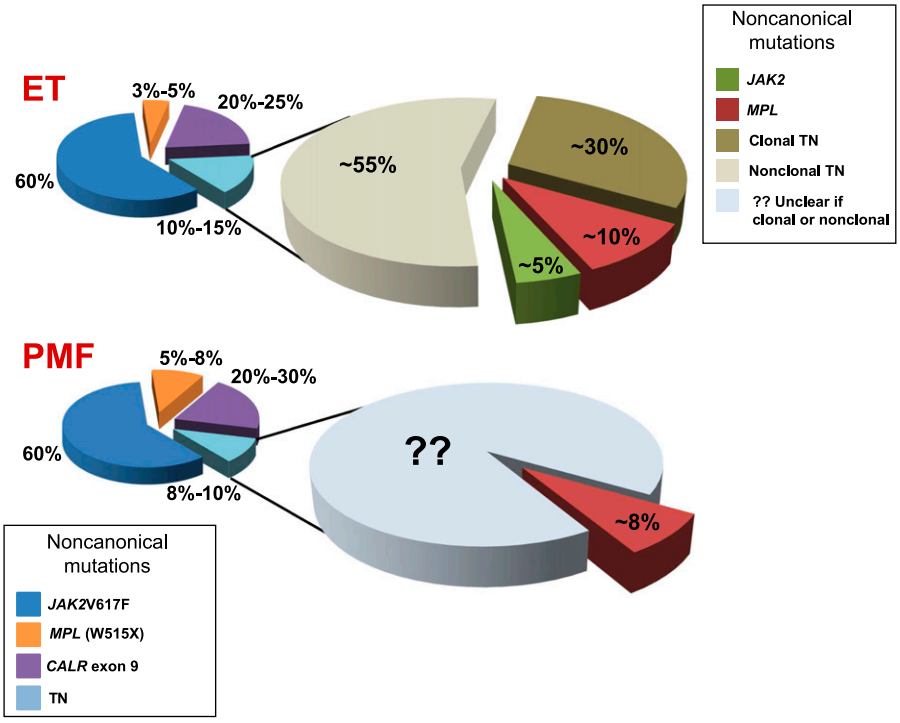
In this issue of *Blood*, Milosevic Feenstra et al[1] and Cabagnols et al[2] report the discovery of heterogeneous novel mutations in *MPL* and *JAK2* genes in 5% to 10% of essential thrombocythemia (ET) and primary myelofibrosis (PMF) patients who lacked what are regarded as classical mutations in these myeloproliferative neoplasms (MPNs) and were thereby considered as having a "triple-negative" (TN) disease. The concept of TN ET and PMF patients was developed after the discovery of calreticulin (*CALR*) mutations.[3,4] The term "triple negativity" was first employed for breast cancer patients who had tumors negative for estrogen or progesterone receptor and HER2 mutations, but it is no longer scientifically correct. TN breast cancers have subsequently been shown to harbor pathogenic mutations in several other genes, including *PI3KCA*, *BRCA1*, *BRCA2*, and *PALB2*,which are now of increasing importance in clinical management.[5] The findings in these 2 manuscripts for TN ET and PMF patients are similarly important and raise several questions for both future research and clinical practice.

Milosevic Feenstra and colleagues[1] began by analyzing tumor cells (granulocytes) and control cells (T lymphocytes) from 8 TN ET and PMF patients subjected to whole-exome sequencing (WES); in 1 patient, a novel somatic mutation at codon 204 of *MPL* (S204) was discovered. This finding prompted conventional (Sanger) sequencing of the entire coding region of *MPL* and *JAK2* in an additional cohort of 61 TN ET and PMF patients, identifying 5 new *MPL* mutations, 3 of which were true somatic, 1 was germ line, and 1was not defined because of lack of control tissue, overall accounting for ~10% of TN patients (see figure). Noncanonical (ie, not V617F) *JAK2* mutations were found in 5 of 57 patients; 3 mutations were germ line, and control cells were not available in 2 patients.



The current genetic landscape concerning phenotypic driver mutations in MPN.

Due to a negative, or not informative, family history, the patients with germ-line *MPL* and *JAK2* mutations were considered to have sporadic MPN. These newly discovered *MPL* and *JAK2* mutations were functionally validated, even though they appeared to have milder gain-of-function effects on Janus kinase 2/signal transducer and activator of transcription signaling than canonical mutations.

In the second study, Cabagnols et al[2] investigated an initial cohort of 17 TN patients with ET by WES targeted on *JAK2* and *MPL* using paired granulocyte and T-cell preparations. Previously described mutations in *MPL* codons other than codon 515 were found in 3 patients, and 1 patient had a rare mutation at codon 515 (W>R), 1 had a low *JAK2*V617F allele burden detected, and another had a mutation in a nondriver gene (ie, *SH2B3*). Four of the remaining 11 patients had evidence of clonal hematopoiesis, based on the detection of heterogeneous, nonrecurrent mutations, whereas in 7 patients, a nonclonal thrombocytosis was diagnosed. Only 1 of 26 additional ET patients analyzed by deep sequencing of *MPL* and *JAK2* was found to have a novel *MPL*Y591 mutation (see figure). Functional analyses qualified *MPL*S204P and *MPL*Y591N mutations as weak gain-of-function variants.

In his 1951 seminal paper, Dameshek[6] wrote, "we find it difficult to draw any clear-cut dividing lines; in fact, so many 'transition forms' exist that one may with equal reasonableness call a single condition by at least two different terms." In 2015, a decade from the original descriptions of *JAK2*V617F, the MPNs are defined by an increasingly intricate genetic landscape. The articles by Milosevic Feenstra et al[1] and Cabagnols et al[2] are important because they not only reflect Dameshek's observations and increase that "intricacy" but also illustrate the limitations of some of the tools that are sometimes taken for granted in both clinical practice and research. For example, the finding of an SRSF2 mutation in addition to *MPL*S505N in a sample from the study by Cabagnols et al prompted a thorough review of the clinical case that finally led to a reclassification of the diagnosis to myelodysplasia with thrombocytosis (therefore, be certain of the clinical material you are using and be ready to reinterpret initial findings). Additionally, whereas WES and Sanger sequencing initially failed to identify some of the mutations in the cohort of patients, subsequent deep resequencing identified 2 further patients, 1 with a *JAK2*V617F mutation and another with *MPL*S204P. These are issues also acknowledged by Milosevic Feenstra and coworkers. Because both studies, based on analyses of T lymphocytes, potentially identified true germ-line mutations, the availability of a different control tissue would be interesting and important.

In addition, there are multiple clinical implications of these findings. The fact that some ET and PMF patients were considered TN solely because the conventional diagnostic tools failed to identify noncanonical mutations in the known phenotypic driver genes sounds reasonable; however, more powerful approaches such as targeted deep sequencing (or even Sanger sequencing) of the entire coding regions of *JAK2* and *MPL* might not be easily transferrable into standard clinical practice. Second, the information that a sizable proportion of TN ET patients do not have a clonal disease might help to explain the overall better prognosis associated with the triple negativity in ET; however, this also raises important issues for the management of such patients for whom cytotoxic treatment may not be appropriate at all. In this regard, although these 2 studies focused mainly on samples from ET patients, with too few PMF patients included to draw any conclusions concerning the implications for TN PMF patients, the prospect of further subdividing this poor-prognosis group would be tantalizing, if it were possible. However, it is not clear how to translate even the data regarding clonality, because none of the methods employed to establish a clonal disease in the 2 articles (ie, WES, comparative genomic hybridization array, or X-linked assays) can reasonably be used in daily practice. Furthermore, these data indirectly suggest that the World Health Organization criteria for the diagnosis of ET, and perhaps PMF, are far from being perfect, because they apparently failed to identify specific patterns helpful to distinguish a true ET from a polyclonal disease. Lastly, if the data presented in these 2 articles are substantiated in further patient cohorts, the proportion of TN ET and PMF patients still considered TN remains high; thus, their underlying pathogenesis remains to be identified.

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