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Oncogenic Drivers in Myeloproliferative Neoplasms: From JAK2 to Calreticulin Mutations

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Abstract During the past 10 years, major progress has been accomplished with the discovery of activating mutations that are associated with the vast majority of BCR-ABL negative human myeloproliferative neoplasms (MPNs). The identification in 2005 of JAK2 V617F triggered great interest in the JAK2-STAT5/STAT3 pathway. Discovery in 2006 of mutants of thrombopoietin receptor (TPO-R/MPL) and later on of mutants in negative regulators of JAK-STAT pathway led to the notion that persistent JAK2 activation is a hallmark of MPNs. In 2013, mutations in the gene coding for the chaperone calreticulin were reported in 20–30 % of essential thrombocythemia and primary myelofibrosis patients. Here, we will address the question: what do we know about calreticulin that could help us understand its role in MPNs? In addition to oncogenic driver mutations, certain MPNs also exhibit epigenetic mutations. Targeting of both oncogenic drivers and epigenetic defects could be required for effective therapy.

Keywords Myeloproliferative neoplasms · Calreticulin · JAK2V617F · TPO-R

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Introduction

Myeloproliferative neoplasms (MPN) are a group of chronic hematological malignancies characterized by the overproduction of mature blood cells such as erythrocytes, granulocytes, and/or platelets [1]. Chronic myeloid leukemia (CML) was differentiated early from other MPN following the discovery of Philadelphia chromosome and BCR-ABL1 fusion genes. Over several decades and in the absence of specific molecular defects, other MPN have been grouped in the Philadelphia negative MPN subgroup, with various entities depending on clinical and biological features. Polycythemia vera (PV) is characterized by an increased red cell production, essential thrombocythemia (ET) displays thrombocytosis, while primary myelofibrosis (MF) is characterized by splenomegaly and early bone marrow fibrosis due to scarring induced by highly proliferating myeloid progenitors and pathological stimulation of local fibroblasts. The natural history of MPN is characterized by the risk of thrombotic events and transformation into acute myeloid leukemia (AML), a highly severe evolution associated with a dismal outcome. PV and ET may also evolve towards secondary myelofibrosis, splenomegaly, and pancytopenia. In the past years, the main therapeutic goal has been to prevent thrombotic events through the normalization of peripheral blood cell counts and the use of antiplatelet drugs. The discovery of somatic driver mutations, associated epigenetic regulator mutations, and abnormal signaling pathways has paved the way to a better understanding of MPN pathogenesis and eventually establishing innovative therapeutic strategies [1].

The Cytokine Receptor-JAK-STAT Pathway

The cytokine receptor-JAK-STAT pathway is a key signaling pathway during myelopoiesis. As a consequence, it is no

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surprise to see that this pathway also plays a crucial role in MPN pathogenesis. Cytokines such as erythropoietin (EPO), thrombopoietin (TPO) or granulocyte colony-stimulating factor (GCSF) bind to their respective receptor—EPO-R, TPO-R, or GCSF-R—on progenitor cells to regulate erythropoiesis, megakaryopoiesis, or granulopoiesis. TPO-R and GCSF-R are also expressed by hematopoietic stem cells. The cytoplasmic domains of these receptors are docking sites for Janus kinases (JAK). JAK2 is one of the four JAKs, which contain an N-terminal FERM domain, a Src-homology like domain (SH2), a pseudokinase domain (JH2), and a C-term kinase domain (JH1) [2•]. The FERM and SH2 domains anchor JAK2 to cytokine receptors, JH2 regulates kinase activity, while JH1 is responsible for tyrosine phosphorylation. Following the binding of cytokines to their receptor, a change in receptor conformation takes place so that JAK2 phosphorylates in trans Y1007 and Y1008 in the activation loop of the JH1 domain, thus inducing JAK2 activation. JAK2 phosphorylates cytokine receptors and signal transducers and activators of transcription (STAT) bind to phosphorylated tyrosine residues on these cytokine receptors through their SH2 domains whereupon JAKs can themselves be phosphorylated by JAKs [1]. Phosphorylated STATs can translocate into the nucleus to be part of transcriptional complexes. In addition to activating STATs, JAKs also activate the phosphatidylinositol 3 kinase (PI3K)/AKT and the Ras-MAP kinase-ERK pathways. These three pathways induce survival, proliferation, and myeloid differentiation.

Somatic Mutations

Three Driving Mutations are Responsible for MPN Development

In the last 10 years, three classes of main somatic driving mutations have been identified in MPN, from JAK2 (JAK2V617F), TPO-R (TPO-RW515) to more recent calreticulin (CALR) mutations (Table 1). Overall, these three mutations account for 90–95 % of MPN and are most often mutually exclusive [3••, 4••, 5•]. JAK2 mutations are found in all PV cases and in around 60 % of MF and ET cases. The JAK2 V617F mutation accounts for 97 % of PV cases, while JAK2 exon 12 mutations around K539 are found in the 3 % remaining cases of PV [6–10]. JAK2 V617F arises in exon 14 of JAK2 on 9p chromosome. The guanine-to-thymidine mutation induces the substitution of valine for phenylalanine at position 617 in the pseudokinase JH2 (JAK homology domain

Table 1 Driving mutations in MPN

	Frequency in MPN	Clinical characteristics[a]	Clinical outcome[a]
JAK2 V617F	PV ≈ 97%	-	Median OS (PV) ≈ 13 years [50]
	ET ≈ 60%		Median OS (ET) ≈ 20 years [50]
	MF ≈ 60%		Median OS (MF) ≈ 5-9 years [49, 50]
Exon 12 JAK2 mutations	PV ≈ 3%	Younger patients, isolated erythrocytosis [75], WBC and platelets counts are often normal [75]	Compared to JAK2V617F PV, similar thrombotic and transformation risks [75]
TPO-R W515L/K/A	PV≈ 0		
	ET ≈ 1%	ET: increased platelet count, decreased Hb [50]	ET: no impact on outcome
	MF ≈ 5%	MF: younger, lower WBC [50], anemia more frequent	MF: Similar outcome compared to JAK2V617F MF [49, 50]
CALR	PV ≈ 0		
	ET ≈ 25%	ET: male and younger patients, increased platelet count, lower Hb levels [41, 50]	ET: low thrombotic risk, no impact on OS ≈ 20 years [50]
	MF ≈ 30%	MF: younger patients, lower WBC, higher platelet count [49, 50]	MF: improved outcome (median OS ≈ 14 years) for type I CALR mutations [50, 51]
Triple negative	PV ≈ rare cases		
	ET ≈ 10 to 15%	ET: younger age, lower Hb, lower WBC [50]	ET: lower thrombotic risk, no impact on outcome [50]
	MF ≈ 10 to 15%	MF: lower Hb [49, 50]	MF: poor outcome (median OS≈2.5 years), higher transformation risk [49, 50]

OS overall survival, *Hb* hemoglobin level, *WBC* white blood cell counts, *PV* polycythemia vera, *ET* essential thrombocythemia, *MF* myelofibrosis

[a] For exon 12 JAK2, CALR, TPO-R or triple negative mutations, clinical characteristics and outcome were compared to JAK2V617F cases

2 or pseudokinase) domain [2•]. This mutation rigidifies the α helix C in the JH2 domain to promote the transphosphorylation of JH1 [2•]. The precise mechanism of conformational change in JH2 induced by V617F is represented by aromatic interaction between F617, F595, and F594 in the helix C of the JH2; an aromatic residue is required both on helix C and at position F595 for constitutive activity [11, 12]. Tyr 1007 and Tyr1008 in JH1 (kinase domain) are phosphorylated, so that JAK2V617F is constitutively active in the absence of cytokine [13]. Of note, JAK2V617F needs the presence of cytokine receptors—especially TPO-R—to be constitutively active, as the deletion of TPO-R significantly reduces MPN phenotype in vitro and in vivo [14]. The transphosphorylation of JAK2 induces constitutive activation of STATs in the absence of cytokine, thus contributing to the cytokine-independent growth of hematopoietic progenitors in methylcellulose assays. Amongst various STATs, STAT5A and STAT5B play a crucial role in MPN pathogenesis, as deletion of both Stat5 a/b genes abrogates the PV phenotype in JAK2V617F mouse models [15, 16]. Noteworthy, STAT5 DNA binding sites differ between cytokine activated and constitutively activated STAT5 because constitutively active STAT5 binds to low affinity γ interferon activated sites [17]. Constitutively activated STAT5 also recruits TP53 in a transcriptional complex at specific STAT5-TP53 genomic promoters to induce the expression of specific genes such as miR-28, which targets and downregulates TPO-R expression [18, 19]. As such, target genes of the STAT5-TP53 complex differ from target genes of STAT5 or TP53 taken alone. These two mechanisms contribute to the development of a specific gene expression profile in MPN. On the contrary, STAT1 and STAT3 impact on the specific MPN which arises following JAK2 V617F mutation. STAT1 promotes an ET-like phenotype possibly through increased levels of Interferon γ because deletion of Stat1 impairs megakaryopoiesis and promotes erythropoiesis in a conditional JAK2 V617F murine model [20]. Conversely, Stat3 deletion increases thrombocytosis in conditional JAK2 V617F mice [21]. PI3 kinase activation by JAK2 V617F also triggers replication defects with an altered intra-S checkpoint response and a replication fork stalling [22]. JAK2 V617F mutation results in a monoclonal population in human MPN, and the presence of JAK2 V617F in a single LSK cell is sufficient to induce MPN in a murine model [23]. Whether JAK2 V617F progenitor and stem cells display a growth advantage over wild-type hematopoietic stem and progenitor cells has been a matter of debate [23–25].

Mutations of TPO-R can be found in around 5 % of MF and 4 % of ET patients [26, 27]. These mutations occur mainly at position W515 in an amphipathic RWQFP domain of the human receptor at the junction between the transmembrane and the cytoplasmic domains, which is required for maintaining the TPO-R inactive in the absence of ligand [28]. While W515 prevents TPO-R dimerization in the absence of TPO, the substitution of tryptophan for leucine, lysine, arginine, serine, or others induces constitutive TPO-R dimerization and activation of TPO-R [29]. In vivo pathogenicity of TPO-R W515 mutants requires Tyr 626 of the cytosolic domain that links the receptor to MAP-kinase and STAT3 [30]. Another mutation that is found in familial and rare sporadic ET is the transmembrane S505N human TPO-R mutation, which induces an active orientation of the transmembrane domain of TPO-R [29].

In 2013, a third driver mutation, occurring in the gene CALR was discovered in JAK2 wild type (WT) and TPO-R WT MPN and accounts for 20 to 30 % of ET and MF [3••, 4••].

CALR

What Do We Know About CALR?

CALR encodes calreticulin, a multifunctional 55 kDa chaperone protein localized in the endoplasmic reticulum (ER), that is involved in calcium retention and protein folding, as well as in immune responses, by the ability of CALR to be translocated to the cell surface in apoptosing cells [31–34]. Although various CALR mutants (over 50) in exon 9 have been described, the most frequent ones are a deletion of 52 base pairs or an insertion of 5 bases pairs in exon 9, denoted del 52 (type 1) and ins 5 (type 2) [3••]. All detected mutations induce a frameshift to the +1 frame, thus resulting in the same novel positively charged sequence. Contrary to the wild-type CALR, which contains at the C-terminus (coded by exon 9) several stretches of negatively charged amino acids (KDEE) involved in binding calcium with low affinity and high capacity and a KDEL C-terminal motif, the CALR mutants contain the novel positive amino acids and lack the KDEL ER retention motif at their C-terminus [3••]. Notably, granulocytes with CALR mutation also display a JAK2 activation signature and an enrichment of STAT5A/B target genes, thus showing that common pathways trigger MPN pathogenesis irrespective of JAK2 or CALR mutation [5•]. CALR mutated patients also exhibit an increased expression of EZH2 and SUZ12, two members of the polycomb repressive complex 2, suggesting that epigenetic regulations might take place in CALR mutated granulocytes compared to normal granulocytes [5•].

Of interest, both the wild type and MPN mutants of CALR exhibit intact N-terminal and Proline-rich (P) domains (Fig. 1). While CALR has been classically described as binding high mannose immature N-glycosylated proteins of the ER that are also monoglucosylated, there are both glycan-dependent and glycan-independent interactions with protein substrates [35–37]. The N-terminal domain is involved in both types of

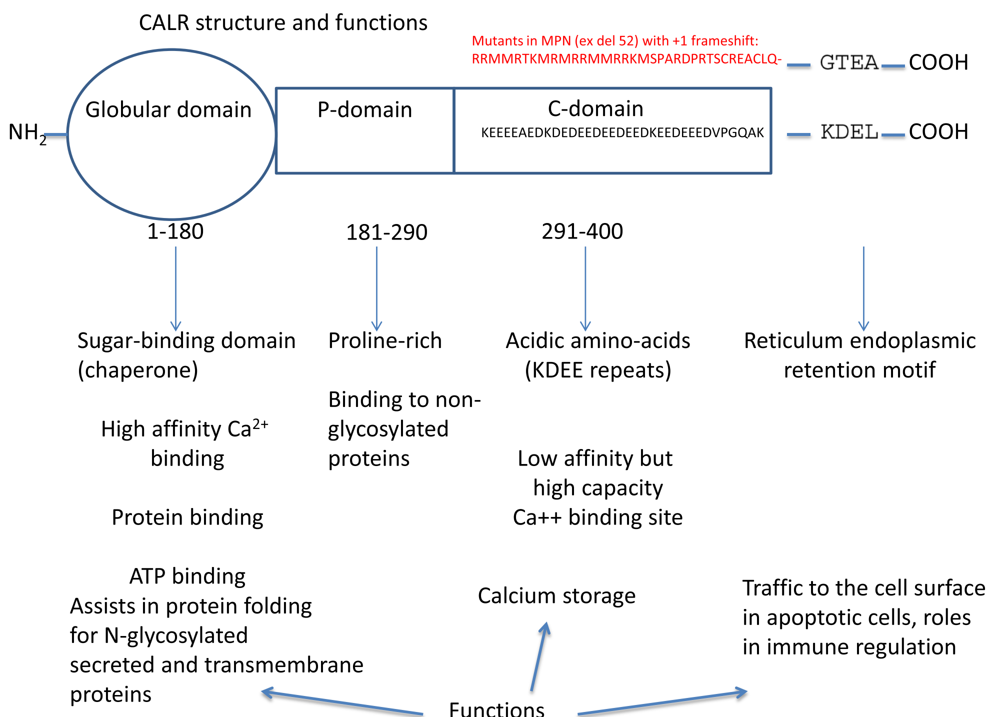

Fig. 1 Calreticulin (CALR) protein structure and functions. CALR is located in the lumen of the endoplasmic reticulum. It contains a globular N-terminal domain that is responsible for sugar binding, a proline-rich (P) domain (also denoted the P-arm), which attracts the ERp57 chaperone and promotes peptide binding, and a C-terminal domain coded by exon 9 which contains a high frequency of negatively charged aspartic and glutamic amino acids that are involved in low affinity high capacity calcium binding. At the C-terminus of the wild-type sequence (*black*), there is a KDEL ER retention sequence. In contrast, in the MPN-associated CALR mutants, the C-terminal sequence is changed for a sequence that contains several positively charged amino acids like arginine and lysine, and at the C-terminus, there is no KDEL ER retention sequence. Certain functions of CALR are depicted, from the classical chaperone function linked to binding monoglucosylated high mannose Asn glycosylated proteins to novel functions, such as exposure on the cell surface on apoptosing cells and stimulation of immunogenic cell death. The mechanism by which wild-type CALR can be translocated to the cell surface is not known

interaction, but for the latter, the P domain contributes to the non/deglycosylated proteins [37]. Another ER chaperone, ERp57, forms complexes with the P domain of CALR [37]. The X-ray structure of the globular lectin binding domain of CALR has been solved, revealing a peptide binding area, and indicating a multi-molecular (peptide and sugar binding) mechanism [38, 39]. A long channel on CALR formed by a concave β-sheet was shown to bind a tetrasaccharide [38].

Preliminary Evidence Linking CALR Mutants to the JAK-STAT Pathway and MPN Phenotype

Transplantation of murine bone marrow transduced with retroviruses coding for del 52 and ins 5 CALR mutants results in an MPN phenotype with thrombocytosis after week 13, which can be propagated upon serial transplantation [40]. Subsequent work demonstrated that the allele burden of CALR mutants appears to be significantly higher in ET patients with CALR mutations than in JAK2 V617F ET patients [41]. Given that CALR mutated patients are younger, these differences suggest that CALR mutants might have a more profound effect on amplifying mutated stem cells, and that the disease penetrates faster in the granulocytic compartment. With one exception, where CALR mutation was found in a PV patient [42], the vast majority of data indicate that CALR mutants are associated with ET and MF and are mutually exclusive with TpoR and JAK2 mutations [42, 3••]. In one recent report, a patient with CML and thrombocytosis who remained thrombocytotic after anti-ABL treatment was found to have an acquired CALR mutation in a founding clone, where subsequently BCR-ABL was acquired [43]. Treatment with ABL inhibitors targeted the BCR-ABL positive clone but not the CALR mutated clone, and the patient remained thrombocytotic [43]. Thus, CALR mutations can be suspected in CML patients that remain thrombocytotic after ABL inhibition.

The absence of the KDEL from the C-terminus of CALR mutants would predict its localization in different cellular compartments to wild-type CALR which via the KDEL receptor is retrieved and from Golgi and ER-to-Golgi vesicles back to the ER. In one of the initial papers, describing the CALR mutation in MPN indeed the mutants were found to lose co-localization with calnexin, a marker for the ER [3••]. Unpublished evidence from our group where CALR-/- fibroblasts were reconstituted with wild type, del 52 or ins 5 CALR mutants shows also that the mutants CALRs exhibit different localization from wild-type ER and that they are able to leave

the ER (Pecquet et al., unpublished). However, in the other initial report, CALR mutants were shown to continue to be localized in the ER [4••]. In this particular report, fusion proteins between CALR mutants and GFP were used, and such fusions might influence traffic [4••].

Also in the first report, evidence was provided that CALR mutants activate the JAK-STAT pathway [3••]. Retroviral expression of CALR mutants, but not wild-type CALR in the IL3-dependent proB Ba/F3 cell line, led to appearance of cells that grew autonomously and, especially for the del 52 CALR mutant, STAT5 activation could be demonstrated, although weak [3••]. These cells were sensitive to JAK2 inhibitors [3••]. Thus, it appears that CALR mutants can directly or indirectly activate JAK2. This is supported by data from clinical trials where patients with CALR mutations responded to JAK2 inhibitors and to type I interferon, like JAK2 V617F positive MPN [44, 45]; the question of course remains what the precise mechanisms behind this effect might be. In addition, CALR mutants are predicted to profoundly influence calcium homeostasis and reduce calcium reserves in the ER. This, in turn, might mis-regulate nuclear factor of activated T cells (NFAT) activation (de-phosphorylation), which allows NFAT to be translocated to the nucleus and regulate transcription.

Evidence Against a Major Role For JAK-STAT Pathway in the Oncogenic Effect of CALR Mutants

One leukemia cell line, MARIMO, was found to harbor del 61 CALR, which exhibits the same frameshift and is a non-typical, but pathogenic CALR mutation [46]. In these cells, there is no detectable activation of the JAK-STAT pathway, and levels of JAK2 are very low. In contrast, these cells appear to exhibit high MAP-kinase activation. Absence of JAK-STAT activation in the presence of this mutation was interpreted as evidence that JAK-STAT pathway is not key for the oncogenic action of CALR mutants [46]. However, this cell line has been derived from a leukemia patient that previously had ET and was treated with Busulfan and exhibits MYC amplification [47]. Thus, proliferation of MARIMO cells might not need pathologic JAK-STAT signaling.

Differential Association of CALR Type 1 and Type 2 Mutations With Myelofibrosis and Essential Thrombocytemia

The type 1 CALR mutation (del 52) seems to result in signaling at higher levels than the type 2 (ins 5) mutation, based on the first in vitro and in vivo studies [3••, 40]. Perhaps predictably, the type 1 mutation is significantly more frequent in MF than in ET [3••, 48], while the type 2 is more frequent in ET than in MF [48]. This supports the notion that higher pathogenic signaling leads to progression to MF, like in the case of JAK2 V617F [1]. In MF, and contrary to ET, patients with CALR mutated display an improved outcome compared to patients with JAK2V617F mutation (Table 1) [49, 50]. Although type 2 CALR mutation is less frequently associated with MF, when it is, it seems to induce a less favorable evolution than type 1 CALR mutation [51]. One explanation could be that a weaker mutation will require and lead to selection for other mutations, possibly epigenetic; another would be that ins 5 CALR induces a qualitatively different signal than del 52 CALR. Further studies are required to settle this issue.

Other Mutations Than Phenotypic Drivers Can Affect MPN Outcome

Additional mutations are also found in MPN in association with one of these driving mutations. These mutations may affect epigenetic regulators such as Ten-eleven translocation 2 (TET2), DNMT3A, ASXL1, EZH2, or genes involved in apoptosis such as TP53 [52•]. Genomic mutations have been sequentially looked for in granulocytes of MPN patients using targeted next-generation sequencing [52•]. Surprisingly, most mutations were already found at diagnosis, and only two somatic mutations occurred over a cumulative period of 133 patient-years [52•]. MPN cells turn out to be highly stable from a genomic point of view [52•]. The clinical outcome may therefore be predicted from somatic mutations found at diagnosis [52•]. In particular, the more mutations at diagnosis, the poorer long term outcome [52•]. The coexistence of various mutations at diagnosis drives clonal heterogeneity and evolution during disease natural history [52•, 53].

Ten-eleven translocation TET2 mutations were first described in myeloid malignancies in 2009, notably in MPN patients with heterozygous JAK2 V617F mutations [54]. The TET2 enzyme catalyzes the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine and subsequently to 5-formylcytosine and 5-carboxylcytosine and initiates DNA demethylation [55]. TET2 mutations may occur before or after driving mutations, thus giving rise to the coexistence of various clones at the same time [52•, 56]. Loss of TET2 induces an increased proliferation of hematopoietic stem cells (HSC) and confers to JAK2 V617F HSC a competitive advantage over JAK2 V617F/TET2 wild type HSC [56–58]. Whether TET2 mutations are associated with an adverse outcome and a higher risk of transformation is controversial [52•, 59]. Of note, the order of occurrence between TET2 and JAK2V617F mutations matters and triggers distinct biological and clinical effects [56]. Other mutations have been detected in MPN patients. Mutations of DNMT3A—a DNA methyltransferase—have also been found in 5 % of MPN patients [52•, 60]. EZH2 is a histone methyl transferase and a member of the polycomb repressive complex 2, which induces the formation of heterochromatin and participates in gene repression. EZH2 loss of function mutations have been found in a few

MPN cases (3 %) [52•, 61]. Other genes coding for chromatin modifiers have been described in MPN, such as ASXL1 [62]. Other mutations have been found in AML transformation, especially isocitrate dehydrogenase 1 and 2 (IDH1/2) [63]. Of note, IDH1 mutations induce severe epigenetic defects [64].

TP53 mutations are highly associated with transformation into AML [65]. Similarly, amplification of a region located on 1q region containing MDM4— encoding a p53 binding protein and inhibitor—is associated with AML transformation [65]. When comparing paired chronic phase and transformed MPN, an increase in TP53 variant allele frequency is observed [53]. Chronic MPNs can be associated with heterozygous TP53 mutations, while acquisition of homozygosity of TP53 mutation, or mutation of the other TP53 allele results is associated with secondary acute myeloid leukemia [52•]. The transplantation of TP53-/- JAK2 V617F bone marrow cells in mice induces AML [53]. Of note, leukemic cells develop from the megakaryocyte-erythroid progenitor cell population [53]. Leukemic transformation results from the expansion of the TP53 mutated clone and the loss of the WT allele [53].

Therapeutic Implications

The discovery of JAK2 V617F has paved the way to JAK2 inhibitors. Ruxolitinib is an ATP competitor and a potent JAK1 and JAK2 inhibitor [66]. In two landmark clinical papers in 2012, ruxolitinib induced a spleen size reduction and a relief of clinical symptoms in patients with myelofibrosis compared to placebo (COMFORT I trial) or best available therapy (COMFORT II trial) [67••, 68••]. These trials led to the approval of ruxolitinib by the US and European administrations for the treatment of myelofibrosis. More recently, the RESPONSE trial demonstrated that ruxolitinib was superior to standard therapy to reduce hematocrit and reduce splenomegaly in patients with PV [69••]. Of note, the effectiveness of ruxolitinib is not restricted to JAK2 V617F MPN and has also been observed in JAK2 WT MPN. Whether ruxolitinib will reduce AML transformation remains to be proven. While ruxolitinib obviously improves the MPN armamentarium in MPN therapy, the impact on allele burden is modest, a reduction of around 12 % after 32 weeks of therapy in the RESPONSE trial [69••]. The very fact that ruxolitinib is not JAK2 V617F specific and inhibits JAK2 WT may participate in this limited activity. Beyond, other JAKs, JAK3 and TYK2 may also compensate for the inhibition of JAK1 and JAK2 activities [70]. Lastly, the microenvironment and especially stromal cells may participate in the JAK inhibitor resistance through the release of cytokines such as interleukin 6, fibroblast growth factor or CXCL10, which may contribute to the stability of JAK2 phosphorylation [71].

To improve JAK2 inhibition, various strategies are being tested. Synthesis of a JAK2V617F specific inhibitor would likely improve JAK2 inhibition in MPN cells while sparing healthy JAK2WT hematopoietic stem and progenitor cells. The addition of a PI3K inhibitor to ruxolitinib may improve JAK inhibitor efficiency [72, 73]. Similarly, use of HSP inhibitors together with JAK inhibitors decreases JAK2 phosphorylation and improves clinical signs of the disease including fibrosis [74] .

Conclusion

While the molecular defects of BCR-ABL negative MPNs have remained unsolved for almost a century, the past 10 years have seen the discovery of various mutations in MPN, translating into the development of JAK inhibitors in these diseases. While being a substantial improvement in patients' care and quality of life, these molecules nonetheless do not provide a cure to patients with MPN. A new player recently emerged in a substantial fraction of MPNs, CALR which upon insertions and deletions in its last exon leads to novel proteins that drive ET and MF, but apparently not - or very rarely - PV, and appear to support pathologically persistent JAK2 and STAT5 activation. Whether mutated CALR proteins are secreted or act only intracellularly remains to be determined. Further studies will have to establish the mechanistic links between these mutants, their traffic and potential signaling leading JAK-STAT activation, as well as their roles in hematopoietic stem and progenitor cells, especially megakaryocytes. Understanding how all these mutations alter or synergize with the epigenetic configuration will be key issues to improve MPN therapy.

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Compliance with Ethics Guidelines

Conflict of Interest Xavier Cahu declares no potential conflicts of interest. Stefan N. Constantinescu is a board member for Dafra Pharma, Belgium, Personal Genetics, Romania, Novartis, Myelofibrosis Board, and Shire, Ad-Hoc Anagrelide; is a consultant for Novartis, Teva, and Shire; reports a grant from Aventis; and payment for the development of educational presentations including service on speakers' bureaus and travel/accommodations expenses covered or reimbursed from Teva, Shire, and Novartis.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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