Introduction

In the landmark perspective written by William Dameshek in 1951, the concept of “myeloproliferative disorders” as a related group of diseases was proposed. In this important paper, Dameshek alluded to the presence of a shared “myelostimulatory factor,” which may have explained overlapping clinical and laboratory features in his “myeloproliferative disorders.” Some 55 years later, the JAK2 V617F mutation was discovered in patients with essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF). Following this discovery, mutations of the thrombopoietin receptor (TPO-R) MPL were reported in a minority of ET and MF patients. In 2013, the first descriptions of the calreticulin (CALR) mutations were reported in patients with JAK2 and MPL-negative ET or MF. These 3 mutations, referred from here on as “driver mutations” have clearly enhanced diagnostic capability. Recent reports, particularly focused on CALR, have shed further light on contributions to disease pathogenesis. The driver mutational profile also influences prognosis, including vascular complication rates in ET and longevity in MF. Further, next-generation sequencing (NGS) techniques have allowed for a more nuanced understanding of prognosis. This review provides an overview of driver (JAK2/CALR/MPL) and other frequently reported mutations in myeloproliferative neoplasms (MPNs), discussing their prognostic and therapeutic implications, and role in routine clinical practice.

Driver mutations

JAK2 mutations

The JAK family of enzymes includes JAK1, JAK2, JAK3, and TYK2. These molecules attach to the cytosolic domains of cytokine receptors, and are essential for cytokine and growth factor signaling. JAK2 is the only member capable of mediating signaling through the 3 myeloid receptors (erythropoietin/MPL/granulocyte colony-stimulating factor receptor).

The JAK2 V617F mutation is the result of a guanine to thymine change at nucleotide 1848 of exon 14 of the JAK2 gene, which leads to a single amino acid substitution from valine to phenylalanine at codon 617. The mutation results in dysregulated ligand-independent JAK2 kinase activity, due to its localization within the pseudokinase (JH2) domain, which negatively regulates activity of the kinase (JH1) domain. JAK2 V617F mediates the activation of downstream signaling through STATs (STAT5, STAT3, and STAT1), extracellular signal-regulated kinase/mitogen-activated protein kinase, and phosphatidylinositol 3-kinase/AKT/mammalian target of rapamycin pathways, resulting in uncontrolled myeloproliferation. Furthermore, JAK2 V617F has the capability to act as an epigenetic modifier, and has been reported to phosphorylate the protein arginine methyltransferase (PRMT5) with a much greater affinity than wild-type (WT) JAK2, leading to a decreased methyltransferase activity and resultant myeloproliferation.

Uniparental disomy at the JAK2 locus on chromosome 9 results in homozygosity of the JAK2 clone, and is responsible for the allelic variation in JAK2 and phenotypic differences in JAK2-driven MPNs.

Recently, novel mutations of the JAK2 gene have been reported in a subset of patients with ET and primary myelofibrosis (PMF) who were negative for the JAK2 V617F mutation. It should be noted...
that the methods used to identify these mutations, such as whole exome sequencing (WES), are not currently available for adoption in routine daily practice.

**MPL mutations**

Located on chromosome 1p34, the MPL gene encodes for the TPO-R MPL, which signals through JAK2 and is considered essential for megakaryopoiesis. The first somatic MPL, W515L, mutation was described in 2006, with a guanine to thymine change at nucleotide 1544, resulting in a tryptophan to leucine substitution at codon 515. Several other gain-of-function mutations involving W515 have also been reported to occur in exon 10 of the MPL gene, resulting in the substitution of tryptophan at codon 515 to lysine, arginine, or alanine, and corresponding to W515K, W515R, and W515A mutations, respectively. MPL mutations have been reported in 4% of patients with ET and 5% to 9% of patients with MF. Novel MPL mutations had been recently identified by utilizing WES of granulocytes obtained from patients with ET and MF who lacked JAK2 and MPL mutations. The ability to incorporate these novel mutations in diagnostic testing to demonstrate clonality is not yet feasible in clinical practice.

**CALR mutations**

CALR is a protein that resides in the lumen of the endoplasmic reticulum (ER), where it functions as a molecular chaperone for many glycoproteins, assisting in their folding and contributing to calcium homeostasis. CALR is also found outside of the ER where it has been implicated in a variety of biological processes, including proliferation and apoptosis. CALR has 3 main structural and functional domains: an N-terminal lectin-binding domain, a proline-rich P domain, and a C-terminal acidic domain that contains multiple calcium-binding sites/KDEL necessary for binding to the ER. CALR mutations are typically found in a heterozygous state with either a 52-bp deletion (type 1) or a 5-bp insertion (type 2 mutation) in the last exon encoding the C-terminal amino acid of the CALR protein, representing the most frequent types and found in >80% of all patients with a CALR-mutant MPN.

All CALR genetic variants cause a loss of a sequence of 27 amino acids, leading to a loss of most of the C-terminal acidic domain and the KDEL sequence necessary for the function of the CALR protein and binding to the ER. The exact mechanism by which CALR mutations caused an MPN phenotype was not elucidated until recently, when 3 groups reported a novel signaling mechanism, whereby a mutant CALR protein constitutively activated receptor signaling through an abnormal interaction with the TPO-R (MPL). It was demonstrated that the interaction of CALR mutant proteins and the TPO-R directly led to dimerization and activation of JAK2 kinase. Interestingly, the extracellular domain of Tpo-R is reported to be essential for the TPO-independent activation by CALR mutant protein; however, additional studies are needed to determine the structural details of CALR mutants/TPO-R interaction. These data are particularly relevant because it was recently reported that TPO-R antagonists selectively deplete MF hematopoietic stem/progenitor cells and may represent a potentially new approach for treatment of MF patients.

**Driver mutations and their prognostic implications**

**JAK2 mutations and PV**

Because ~95% of PV patients have JAK2 V617F mutations and 4% harbor JAK2 exon 12 mutations, comparisons between JAK2 positive and negative patients are not plausible. The presence of CALR mutations in 2 cases of JAK2-negative PV has been reported, but this appears to be an exceptional circumstance. Although those with JAK2 exon 12 mutations may have higher hemoglobin (Hb), and lower platelet and leukocyte counts when compared with those with JAK2 V617F mutations, clinical outcomes do not differ, given similar incidences of thrombosis, MF, acute myeloid leukemia (AML), and death. However, associations between the JAK2 V617F allelic burden, clinical phenotype, and disease outcomes in PV patients have been reported. In particular, homozygous allele burden has been associated with older age, male sex, pruritus, and splenomegaly; associations between homozygous or increasing allelic burdens and thrombosis (arterial and venous), as well as MF transformation have also been suggested. Another well-recognized phenotypic association with JAK2 V617F includes hepatic and portal vein thrombosis, but affected patients present a unique exception with regard to demographics, clinical phenotype, and allelic burden, because this complication is often observed in younger women, with either a masked phenotype, or lower leukocyte counts and lower allelic burdens. Mutations involving MPL, JAK2 exon 12, and CALR are infrequently identified relative to JAK2 V617F in patients presenting with abdominal vein thrombosis.

**Driver mutations and ET**

In the initial descriptions, it was reported that CALR-mutated ET patients had a lower Hb and leukocyte count, and a higher platelet count at diagnosis, compared with those with the JAK2 V617F mutation ($P < .0001$). Further, CALR-mutated ET patients had a lower risk of thrombosis ($P = .003$) and longer survival ($P = .04$) compared with those with the JAK2 V617F mutation. Nangalia et al also observed higher platelet counts in CALR-mutated ET patients, compared with those with JAK2 mutations ($P < .001$); however, although a higher rate of post-ET-MF transformation was reported in CALR-mutated patients vs JAK2-mutated patients ($P = .03$), there were no differences in survival rates by mutation. Subsequently, large series have confirmed lower thrombosis rates in CALR-mutant ET patients, compared with those with JAK2-mutant ET; interestingly, no CALR-mutant ET patient evolved to PV, whereas the 10-year risk was near 30% in those with JAK2-mutated ET.

Despite consistent reports of a lower thrombosis rate in CALR-mutant ET patients, there appears to be little utility in incorporating CALR into the International Prognostic Score of Thrombosis in ET (IPSET)—thrombosis score, which places weight on the presence of the JAK2 V617F mutation. One possible explanation is the co-segregation of CALR mutations with other lower thrombotic risk features, such as younger age and less frequent history of thrombosis, which are already incorporated into the scoring system.

**Driver mutations and MF**

Although the first descriptions of CALR mutations reported associations with longer survival or a higher rate of post-ET-MF transformation, these associations have been inconsistent. Perhaps, the associations may differ depending on the type of CALR.
Driver mutations and MF

Initial descriptions of CALR mutations suggested that type 1 deletions were frequent in MF compared with ET; as in ET, MF patients with CALR mutations also had lower leukocyte counts and higher platelet counts compared with those with JAK2-mutant MF.7 Longer overall survival was also noted in this initial study for those with CALR-positive MF, compared with those with a JAK2 or MPL mutation.9

In a study of 617 patients, 64.7% carried JAK2 V617F mutations, 22.7% had CALR mutations, 4% had MPL mutations, and 8.6% had a TN profile.17 Consistent with prior studies, at presentation, CALR-mutated patients were younger, with lower leukocyte counts, higher platelet counts, and lower risk groupings; TN patients were older, with lower Hb, lower platelet counts, and higher risk groupings. During the course of follow up, CALR-mutant patients had a lower cumulative incidence of developing anemia (Hgb <10 g/dL), thrombocytopenia (<100 x 10^9/L), and marked leukocytosis (>25 x 10^9/L), and a longer interval to the development of large splenomegaly (>10 cm below the left costal margin) compared with other mutational subgroups.17 Thrombosis rates were lower in those with CALR mutations (13.6%) compared with those with JAK2 V617F mutations (18.3%; P = .021). The 10-year cumulative incidence of blast transformation was highest in TN patients (34.4%), compared with those with JAK2 V617F (19.4%; P = .043 for comparison), MPL (16.9%), and CALR (9.4%; P = .016 for comparison) mutations. Mutational profile also independently impacted median overall survival, which was 17.7 years for CALR-mutated patients, 9.2 years for JAK2 V617F-mutated patients, 9.1 years for MPL-mutated patients, and 3.2 years for TN patients. When adjusting for age, CALR-mutant patients still have improved overall survival compared with those with JAK2 mutations or TN MF.17 The impact of the JAK2 V617F allelic burden was not examined in this study; previous findings have been inconsistent, although lower allelic burdens have been correlated with a worse outcome.30

A study of 428 PMF patients also confirmed the prognostic impact of the driver mutational profile.38 Leukemia-free survival was worse in those with TN PMF compared with CALR, JAK2, and MPL-mutated MF; CALR-mutated patients had a lower risk of blast transformation compared with those with TN and JAK2-mutated status. TN PMF patients also had the shortest median survival (2.3 years) compared with those with CALR (15.9 years), JAK2 (5.9 years), or MPL (9.9 years) mutations. Survival was better in patients with type 1 CALR mutations compared with type 2 CALR (P = .03) mutations or JAK2 (P < .0001) mutations.38 Recently, a meta-analysis of 6 studies (n = 1381), including a diverse PMF patient population, confirmed the prognostic impact of CALR with improved survival compared with those with JAK2 mutations, but only in the non-Asian population.41 It was hypothesized that the lack of survival benefit in Asian patients could be due to a higher prevalence of type 2 CALR mutations in Asian patients.41 In keeping with studies of this rare population, the differential impact on MF outcomes between type 1 and type 2 CALR mutations has been inconsistent; a recent study could not identify differences in overall survival in those with type-1–like vs type-2–like CALR mutations.37

Interestingly, the driver mutational profile has less impact on prognosis in those with secondary MF, based on a study of 359 patients with post–PV-MF (n = 194) and post–ET-MF (n = 165).29 Only TN post–ET-MF had a shorter survival compared with CALR-mutated post–ET-MF (P = .01), and there was no difference between other genotypes, including type 1 vs type 2 CALR, JAK2, and MPL-mutated secondary MF.29

Other somatic mutations in MPNs and their prognostic implications

Recent utilization of NGS has allowed for simultaneous profiling of multiple genes and has led to the identification of novel somatic mutations in patients with a variety of myeloid neoplasms, including MPNs, mostly occurring in patients with MF (Table 1).2,8,42-50 These somatic mutations can involve genes in the spliceosome machinery (SF3B1, U2AF1, and SRSF2), as well as genes encoding for several epigenetic modifiers (TET2, DNMT3A, IDH1/2, EZH2, and ASXL1). Even though reported somatic mutations lack specificity, because they can be found in a broad range of myeloid neoplasms, there is evidence to suggest that the identification of certain nondriver mutations in MPNs patients is associated with greater risk of disease progression or shortened survival.

Other somatic mutations in ET and PV

Genetic complexity extends beyond the presence of driver mutations in ET and PV. WES of 48 PV and 62 ET samples identified a median of 6.5 mutations per patient in each group (compared with 13 in MF).8 Albeit at low frequencies, in PV, the most commonly mutated, nondriver genes included TET2, followed by DNMT3A. In ET, the most commonly mutated, nondriver genes, also at low frequencies, included DNMT3A, TET2, and ASXL1.8 Interestingly, the order in which mutations are acquired appears to influence clinical features. Through genotyping hematopoietic colonies or using NGS, it was suggested that those patients who acquired JAK2 V617F prior to TET2 were younger, more likely to present with PV than ET, and had higher thrombosis rates, when compared with patients who acquired TET2 mutations prior to JAK2 V617F.51 This study also suggested that mutant progenitors from “JAK2-first” patients were more sensitive to JAK inhibition compared with “TET2-first” patients.51

Recently, the prevalence and relevance of somatic mutations was reported in a different cohort, including 133 PV and 181 ET patients.52 In PV, 44% were reported to have mutations, including 29% with 1 mutation, 14% with 2 mutations, and 1% with 3 mutations. The most common mutations involved TET2 (18%), ASXL1 (11%), SH2B3 (5%), and SF3B1 (3%). The number of mutations impacted overall and MF-free survival; and the hazard ratio (HR) was 2.6 and 13.7, respectively, for those with 2 mutations, compared with 1.7 and 5.1 for those with 1 mutation. In a multivariable analysis, SRSF2 and RUNX1 affected overall survival, IDH2 and RUNX1 impacted leukemia-free survival, and ASXL1, IDH2, RUNX1, KIT, and SETBP1 predicted fibrotic progression.52 In ET, 46% of patients...
had somatic mutations; the most common mutations involved TET2 (13%), ASXL1 (11%), DNMT3A (6%), SF3B1 (5%), CEBPA (4%), and TP53, SH2B3, EZH2, and CSF3R (2% each). The number of mutations (HR, 6.6 for 3 mutations and HR, 2.2 for 1 or 2 mutations) impacted overall survival, but not leukemia or MF-free survival. In multivariable analysis, EZH2 and SF3B1 mutations impacted survival. 52

At present, there is little evidence to suggest the incorporation of testing for nondriver mutation in routine clinical care of patients with ET or PV.

Other somatic mutations and their prognostic implications in MF

In order to clarify the prognostic relevance of nondriver mutations and their impact on survival, an international collaborative project analyzed the outcome of 879 patients with PMF and known mutational status. 53 ASXL1 mutations correlated with constitutional symptoms, leukocytosis, and $\geq 1\%$ circulating blasts; SRSF2 mutations correlated with older age; and EZH2 mutations associated with $\geq 1\%$ circulating blasts. Patients with ASXL1, EZH2, SRSF2, or IDH mutations were at risk for premature death or leukemic transformation. Patients with any one of these mutations are considered to have a “high molecular risk” profile (HMR). However, only ASXL1 mutations remained significantly associated with survival in the context of the International Prognostic Scoring System (IPSS). 53 Interestingly, it was subsequently demonstrated that the number of mutations also matters, because the presence of 2 or more mutations predicted for worse outcomes; the reported median survival was 12.3 years for patients without a mutation compared with 2.6 years for those with 2 or more mutations. 54

Another report on comprehensive mutational screening of 104 genes by NGS at diagnosis and during follow up (N = 197) demonstrated the presence of somatic mutations in 90% of patients, and 37% carried somatic mutations other than JAK2 V617F or CALR. The presence of $\geq 2$ somatic mutations significantly reduced overall survival and increased the risk of AML transformation. Somatic mutations with loss of heterozygosity in TP53 were strongly associated with leukemic transformation. 55

### Table 1. Frequency of molecular mutations in classical MPNs

<table>
<thead>
<tr>
<th>Affected pathways</th>
<th>Mutation</th>
<th>PMF (%)</th>
<th>PV (%)</th>
<th>ET (%)</th>
<th>Localization/relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine signaling</td>
<td>JAK2V617F</td>
<td>50-60</td>
<td>95</td>
<td>50-60</td>
<td>Chromosome 9p24. Most frequent gain-of-function mutation in PV$^{2-5}$</td>
</tr>
<tr>
<td></td>
<td>JAK2 exon 12</td>
<td>—</td>
<td>3-4</td>
<td>—</td>
<td>A gain-of-function mutation, although outside the auto-inhibitory domain of JAK2. Not found in ET/PMF, can be present in post-PV-MF</td>
</tr>
<tr>
<td>MPL</td>
<td>9</td>
<td>—</td>
<td>4</td>
<td></td>
<td>Chromosome 1p34. A gain-of-function mutation$^6$</td>
</tr>
<tr>
<td>CALR</td>
<td>20-25</td>
<td>—</td>
<td>20-25</td>
<td></td>
<td>All described mutations are either indels in the last exon encoding for the C-terminal amino acid of CALR protein (type 1: 52 bp deletion; type 2: 5 bp insertion are the most common), resulting in a mutant protein with loss of ER retention signal</td>
</tr>
<tr>
<td>CBL</td>
<td>6</td>
<td>—</td>
<td>Rare</td>
<td></td>
<td>Chromosome 11q23.3. A loss-of-function mutation with loss of inhibition of cytokine signaling due to abrogated CBL ubiquitin ligase activity$^42$</td>
</tr>
<tr>
<td>LNK</td>
<td>Rare</td>
<td>Rare</td>
<td>Rare</td>
<td></td>
<td>Chromosome 12p24. A loss-of-function mutation with resultant loss of LNK-associated negative regulation of cytokine receptor signaling$^44$</td>
</tr>
<tr>
<td>Spliceosomes</td>
<td>SRSF2</td>
<td>17</td>
<td>—</td>
<td>—</td>
<td>SRSF2 mutations are relatively common in PMF, cluster with IDH mutations, and are independently predictive of poor outcome$^50$</td>
</tr>
<tr>
<td></td>
<td>SF3B1</td>
<td>6.5</td>
<td>—</td>
<td>Rare</td>
<td>A spliceosome mutation. Mutually exclusive of other spliceosomal mutations$^{49}$</td>
</tr>
<tr>
<td>Epigenetic modifiers</td>
<td>ASXL1</td>
<td>8-26</td>
<td>2</td>
<td>Rare</td>
<td>Chromosome 20q11.21. ASXL1 encodes a transcription factor, which functions through histone modification. Mutations affecting exon 12 are found mostly in PMF$^{47,48}$</td>
</tr>
<tr>
<td></td>
<td>IDH1/2</td>
<td>4.2</td>
<td>1.9</td>
<td>0.8</td>
<td>Chromosomes 2q33.3/15q26.1. Mutants cause overproduction of 2-hydroxyglutarate, which inhibits TET2/other KG-dependent enzymes. Presence of mutation may be explored therapeutically, similar to the ongoing IDH inhibitor studies in AML</td>
</tr>
<tr>
<td></td>
<td>EZH2</td>
<td>13</td>
<td>3</td>
<td>—</td>
<td>Chromosome 7q35. Mutations lead to loss of epigenetic regulation, and are typically associated with poor outcome in PMF$^{46}$</td>
</tr>
<tr>
<td></td>
<td>TET2</td>
<td>8</td>
<td>10</td>
<td>5</td>
<td>Chromosome 4q24. Loss-of-function mutations resulting in decreased 5-hydroxymethylcytosine, and interfering with cytosine demethylation. TET2 mutation may have an impact on ET vs PV phenotype (see text)</td>
</tr>
</tbody>
</table>

IDH, isocitrate dehydrogenase; Indels, insertions and deletions; KG, ketoglutarate.
The impact of the mutational profile on prognosis has been reported. Among 570 patients, those CALR-mutated/ASXL1-negative patients had a median 10.4-year survival, CALR-mutated/ASXL1-positive or CALR-negative/ASXL1-negative patients had a median 5.8-year survival, and CALR-negative/ASXL1-positive patients had a median 2.3-year survival. Extending on this theme, new prognostic scoring systems incorporating molecular and genetic features of MF have been proposed.29,30

As previously mentioned, the impact of the mutational profile differs in those with secondary MF, including post-ET and post-PV-MF. Those with post-ET-MF were more likely to have ASXL1 and EZH2 mutations, compared with those with post-PV-MF (29% vs 17%, \( P = .011 \) and 10.3 vs 3.6%, \( P = .022 \), respectively). However, in post-PV-MF, there was no association between a single-somatic gene mutation, HMR profile, or number of HMR-mutations and overall survival. In post-ET-MF, only SRSF2-mutated status correlated with shortened survival (\( P = .001; 4.9 \) years vs 14.5 years; mutated vs WT).

**Molecular mutations and therapeutic implications**

Treatment decisions for MPN patients are not yet driven by the presence or absence of MPN-associated molecular mutations, but rather, influenced by MPN subtype, symptom burden, and risk category (Figure 1). Importantly, risk classifications are evolving, and the influence of mutations is increasingly clear (Figure 2).29,30

![Figure 1](image-url)
Figure 2. (A) Evolution in prognostic assessment: ET. Constructed prior to CALR discovery (*). CALR co-segregates with younger age and absent thrombosis risk, and therefore, does not modify IPSET score (**). Unable to predict MF or AML risk (**). (B) Evolution in prognostic assessment: PV. (C) Evolution in prognostic assessment: MF. The latest proposed MF scoring systems incorporate molecular and genetic information in the assessment (*). The GPSS identified very high (3 points) and high-risk karyotypes (2 points), TN status (2 points), JAK2/MPL-mutated (2 points), type-2/type-2-like CALR-mutated (2 points), ASXL1-mutated (1 point), and SRSF2-mutated (1 point), as independent predictors of shortened survival; these variables were included in this score, along with age >60 years.57 Another system, the Mutation-enhanced IPSS (MIPSS), analyzed 986 PMF patients, identifying age >60, constitutional symptoms, Hb <10 g/dL, platelets <200 × 10^9/L, TN status (1.5 points), JAK2-mutated or MPL-mutated (0.5 points), ASXL1-mutated (0.5 points), and SRSF2-mutated (0.5 points) status as significant, adverse indicators.58 CV, cardiovascular; DIPSS, dynamic IPSS; GPSS, genetics-based Prognostic Scoring System; pts, patients.
Hematopoietic stem cell transplant represents the only curative treatment option in MF, but is typically reserved for patients with intermediate-2 or high-risk disease (noting the impact that somatic mutations may have), and will be discussed in detail elsewhere (see Gupta, in this book).

**Mutations and response to therapy in ET and PV**

In ET and PV, there has been renewed interest in using pegylated-interferon (IFN), and pivotal clinical trials are underway to elucidate its role in the upfront treatment of such patients (#NCT01259856 and #NCT01259817). In part, intrigue is related to not only high rates of hematologic responses, but also molecular responses initially reported in JAK2-mutated ET and PV patients. In a phase 2 trial, with a median of 42 months follow up, complete hematologic responses (CHR) and complete molecular responses (CMR) were seen in 76% (18% CMR) of PV and 77% (17% CMR) of ET patients. In patients with PV, there was a sustained decrease in the ablative burden, with a decrease from a median of 64% to 8% in those treated for 60 months. In this study, the presence of somatic mutations impacted outcomes, because a higher proportion of patients who did not achieve CMR (56% vs 30% in those with CMR) had mutations (most commonly, TET2, DNMT3A, and ASXL1). Further, in those with paired samples, clonal evolution was commonly observed in those who failed to achieve CMR. Finally, those with JAK2/TET2 mutations had higher JAK2 ablative burdens, and less significant reductions during the course of therapy compared with JAK2-mutant/TET2-WT patients. Interestingly, a molecular response does not always accompany a hematologic response in PV.

Responses to IFN have also been recently reported in CALR-mutated ET patients. Among 31 patients, 77% achieved a CHR; the median CALR burden was 41% and a molecular response rate of 42% was reported (CMR plus partial MR). In a cohort of CALR-ET patients treated with hydroxyurea or aspirin, the CALR burden remained stable. This study also analyzed the impact of somatic mutations, and demonstrated that those with additional mutations had poorer molecular responses compared with those with CALR alone. Analyses of mutation type also suggested a differential effect from IFN-α on mutated clones, because some patients experienced a decrease in the CALR burden but an increase in the clonal burden of other somatic mutations.

Another novel ET treatment concept involves telomerase inhibition. Among 18 previously treated patients, 89% had a CHR. Responses were seen regardless of the mutational profile, although more pronounced in those with JAK2 mutations, as molecular partial response rates of 88% were reported in this group; mutant allele burdens were also reduced by 15% to 66% in those with MPL or CALR mutations.

**Mutations and response to therapy in MF**

Whereas the driver mutational profile influences prognosis, there is less impact on response to JAK inhibition. In the phase 3 studies of ruxolitinib compared with placebo or best available therapy, there was no statistically significant difference in efficacy measures when comparing JAK2-mutated and WT patients. Subsequently, a letter reported on spleen and symptom responses to the JAK inhibitor, fedratinib, in patients with CALR-mutated MF. The clinical observation of JAK-inhibitor response regardless of mutational profile is supported by recent translational studies revealing an activated JAK2 signaling signature in MPN patients irrespective of mutational profile.

Studies have subsequently examined the potential impact of somatic mutations on response to MF therapy. Using data from the COMFORT-II study, ruxolitinib-treated patients with HMR profiles (presence of ASXL1, EZH2, SRSF2, or IDH1 mutations) were compared with those with low-molecular risk profiles (defined as absence of the aforementioned mutations). Interestingly, there was no difference in rates of spleen volume reduction or symptom relief; further, similar rates of hematoxicity were seen in both groups. The investigators also reported improved survival, regardless of mutational profile, with reduction in the risk of death even in those ruxolitinib-treated patients with HMR profiles compared with best therapy (HR, 0.57). Another study of patients treated with ruxolitinib reported similar spleen responses in patients regardless of JAK2, CALR, MPL, or TN status, but those with 1 or more mutations in ASXL1, EZH2, or IDH1/2 were less likely to have a spleen response. The number of mutations inversely correlated with spleen response and time to treatment discontinuation, with the worst outcomes observed in those with ≥3 mutations.

A study of imetelstat presents an exception to the observations of efficacy irrespective of the MF-driver mutational status. In a pilot study of 33 patients, a complete or partial response was reported in 21%; although not statistically significant, and unlike the study in ET, response rates were 27% in those with the JAK2 mutation vs 0% in those without the JAK2 mutation (P = .3). Other somatic mutations also impacted response rates, because 32% without an ASXL1 mutation responded, compared with 0% (P = .07) among those with an ASXL1 mutation. Those with mutations of SF3B1 or U2AF1 had the highest complete response rates of 38%, compared with 4% in those without these mutations (P = .04).

**Mutational profiles in clinical practice and the future**

It is clear that the driver mutational profile complements the diagnostic approach to MPNs, as discussed. In ET, the presence of CALR typically co-segregates with younger age and absence of a thrombosis history; in this way, perhaps identifying patients at lower risk for vascular complications. The impact of the driver mutational profile on prognosis appears strongest in MF, helping set expectations in the clinic; some MF patients with CALR mutations may enjoy a period of longevity. The availability of several NGS-based myeloid panels from commercial and academic laboratories begets the question as to whether they should be used and incorporated in clinical practice. Accruing evidence suggests that identification of certain somatic mutations provide important prognostic information, particularly in MF. The presence of these mutations may upgrade the prognosis to a higher than expected risk category, which may aid in making treatment recommendations, particularly when considering stem cell transplantation. Ideally, a prospective evaluation of mutational profiles would identify patterns of acquisition of new mutations with disease progression and clonal evolution, assess impact of disease duration and therapy on development/evolution of these mutations, and help clinicians in determining whether or not monitoring allele variance frequency is necessary for all myeloid neoplasm patients. In today’s routine clinical practice, however, NGS will not yet guide routine therapeutic decision making. Use of NGS in ET/PV is less established, and in the authors’ opinion, the results are even less likely to be actionable. Because most panels have the capacity to test multiple genes, they can identify somatic mutations involving signaling molecules, epigenetic regulators, tumor suppressor genes, transcription factors, and splicing factors. Ideally, the identification of such molecular signatures or specific pathway aberrancies will lead to the conduct of
rational clinical trials involving novel, precise, and targeted therapies. Nonetheless, a nuanced awareness of Dameshek’s “myelo-stimulatory factors” signifies a breakthrough in understanding for physicians and scientists, and hopefully changes the course for MPN patients.

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References