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Impact of molecular profiling on the management of patients with myelofibrosis

Irene Pastor-Galán [a], Iván Martín [a], Blanca Ferrer [a], Juan-Carlos Hernández-Boluda [a,b,*]

[a] Hospital Clínico Universitario-INCLIVA, Valencia, Spain
[b] University of Valencia, Valencia, Spain

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ABSTRACT

Myelofibrosis (MF) is a chronic myeloproliferative neoplasm (MPN) characterized by a highly heterogeneous clinical course, which can be complicated by severe constitutional symptoms, massive splenomegaly, progressive bone marrow failure, cardiovascular events, and development of acute leukemia. Constitutive signaling through the JAK-STAT pathway plays a fundamental role in its pathogenesis, generally due to activating mutations of *JAK2*, *CALR* and *MPL* genes (i.e., the MPN driver mutations), present in most MF patients. Next Generation Sequencing (NGS) panel testing has shown that additional somatic mutations can already be detected at the time of diagnosis in more than half of patients, and that they accumulate along the disease course. These mutations, mostly affecting epigenetic modifiers or spliceosome components, may cooperate with MPN drivers to favor clonal dominance or influence the clinical phenotype, and some, such as high molecular risk mutations, correlate with a more aggressive clinical course with poor treatment response. The current main role of molecular profiling in clinical practice is prognostication, principally for selecting high-risk patients who may be candidates for transplantation, the only curative treatment for MF to date. To this end, contemporary prognostic models incorporating molecular data are useful tools to discriminate different risk categories. Aside from certain clinical situations, decisions regarding medical treatment are not based on patient molecular profiling, yet this approach may become more relevant in novel treatment strategies, such as the use of vaccines against the mutant forms of JAK2 or CALR, or drugs directed against actionable molecular targets.

Introduction

Myelofibrosis (MF) is a chronic myeloproliferative neoplasm (MPN) that appears *de novo* (primary myelofibrosis, PMF) or develops from a prior polycythemia vera (PV) or essential thrombocythemia (ET) [secondary myelofibrosis, SMF]. The disease originates from a pluripotent hematopoietic stem cell whose clonal proliferation is accompanied by inappropriate release of cytokines and growth factors which induce bone marrow fibrosis and extramedullary hematopoiesis, usually resulting in splenomegaly [1]. Constitutive signaling through the JAK-STAT pathway plays a fundamental role in its pathogenesis, generally via activating mutations of *JAK2*, *CALR* and *MPL* genes [2]. Additional somatic mutations can frequently be found, mostly affecting genes encoding epigenetic modifiers or spliceosome components [3], and some of which have been associated with a more aggressive clinical course and poor treatment response [4–7].

MF is a rare disease with an estimated incidence of 0.5 and 0.2 new cases per 100,000 population per year for PMF and SMF, respectively [8]. Median age at diagnosis is 68 years, but up to a quarter of patients are under 55 years of age at disease presentation [9]. A third of patients are asymptomatic at diagnosis, while the remainder present with varying degrees of constitutional symptoms, anemic syndrome or abdominal discomfort related to spleen enlargement, among other manifestations. Median survival of MF patients is about 6 years [10], somewhat longer in post-ET MF than in post-PV MF or PMF [9,11]. The main causes of death are leukemic transformation (20% of cases), disease progression, infections, heart failure and vascular complications [10]. Allogeneic hematopoietic cell transplantation (allo-HCT) constitutes the only curative treatment, but the advanced age of MF patients and significant transplant-related mortality have historically limited the use of this procedure to <10% of patients [12,13].

In the current review, we provide an overview of the molecular

* Corresponding author at: Hematology Department, Hospital Clínico Universitario, INCLIVA Research Institute, Department of Medicine, University of Valencia, Avd. Blasco Ibáñez 17, 46010 Valencia, Spain.
E-mail addresses: hernandez_jca@gva.es, Juan.Carlos.Hernandez@uv.es (J.-C. Hernández-Boluda).

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landscape of MF by summarizing the frequency and spectrum of somatic mutations identified in patients with this disease, which underlie its great clinical heterogeneity. We then outline the associations between somatic mutations and the main outcomes, such as risk of thrombosis and acute myeloid leukemia (AML) and overall survival. Finally, we address the role of molecular profiling in therapeutic decision making, with particular reference to transplantation, as well as the impact of molecular abnormalities on response to different treatments.

Frequency of gene mutations in myelofibrosis

Phenotypic driver mutations in *JAK2*, *CALR* and *MPL* genes are detected in 50–60%, 20–30%, and 5–10% of MF patients, respectively [3]. These mutations are mutually exclusive with only infrequent co-occurrence [1–2% of cases] [14]. Only 5–10% of patients, the so-called "triple negative" MF patients (TN-MF), have no mutations in any of the three major driver genes [15–17]. Note that the distribution of phenotypic driver mutations does not differ in patients with prefibrotic or overt PMF [18]. Since PV is almost exclusively JAK2-driven, virtually all cases of post-PV MF are *JAK2* mutated.

JAK2 is critical for normal hematopoiesis and its constitutive activation by acquisition of the exon 14 V617F mutation, located in the pseudokinase domain of the protein, results in trilinear myeloproliferation [19]. The mechanisms determining the MPN phenotype (ET, PV or MF) in patients harboring the $JAK2^{V617F}$ mutation are not fully understood, but several factors including sex, mutation allele burden, germline genetic background and additional somatic mutations seem to be involved [2]. The thrombopoietin receptor (MPL) regulates megakaryopoiesis and platelet production and gain of function mutations in the *MPL* gene, usually located at codon 15, constitutively activate the JAK-STAT pathway. Expression of MPL^{W515L} in mouse models resulted in extreme thrombocytosis, rapid development of bone marrow fibrosis and splenomegaly [20]. CALR is a chaperone protein with a key role in maintaining cell calcium homeostasis owing to its negatively charged C-terminus. *CALR* indel exon 9 mutations lead to a novel C-terminal chain of positively charged amino acids that binds to the extracellular domain of MPL, activating the JAK–STAT pathway [21]. Classical type 1 (a 52-bp deletion; p.L367fs*46) *CALR* mutations are more prevalent than type 2 (a 5-bp insertion; p.K385fs*47) in MF patients [22] and have been shown to induce a higher degree of bone marrow fibrosis and osteogenesis in murine models [23]. Non-type 1 or type 2 *CALR* mutations are categorized as type 1/like and type 2/like variants based on structural similarities to the corresponding classical mutants [24].

In addition to mutations in signaling genes (MPN driver genes), high-throughput next-generation sequencing (NGS) studies have detected somatic mutations in known drivers of myeloid malignancies in a significant proportion of MF patients, as summarized in Table 1 [4,14,25–27]. These mutations can precede the acquisition of the MPN driver mutation or occur subsequently in the same or a different clone [28]. Current evidence suggests that concomitant somatic mutations may cooperate with MPN drivers to favor clonal dominance, influence the clinical phenotype, or promote disease progression [29]. These mutations alter DNA methylation (*TET2*,*DNMT3A*, *IDH1*, and *IDH2*), chromatin modification (*ASXL1*, *EZH2*), messenger RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*), cell signaling cascades (*CBL*, *NRAS*, *KRAS*, *SH2B3*, *NF1*, and *PTPN11*), transcriptional activity (*RUNX1*, *NFE2*), and DNA repair (*TP53*, *PPM1D*). Despite the significant overlap in the frequency and spectrum of somatic mutations in patients with PMF and SMF, some differences have been noted. For example, *SRSF2* and *U2AF1* genes are more often mutated in patients with PMF than SMF [30]. Similarly, *ASXL1* mutations are more common in PMF, and their adverse prognostic impact seems to be circumscribed to this MF subtype [31]. Furthermore, a higher prevalence of mutations in *ASXL1* and *EZH2* genes has been found in patients developing MF after ET than PV [27]. Compared with prefibrotic PMF, high risk mutations (any mutations in *ASXL1, SRSF2, IDH1/2* and *EZH2* genes) are more prevalent in overt

Table 1
Most frequent additional somatic mutations in myelofibrosis.

Biological pathways and genes	Frequency	Mutation acquisition	Type of abnormality	Clinical correlations
DNA methylation				
TET2	10–20%	More often a founding mutation	Loss of function	No impact on prognosis [25,99]
DNMT3A	5–10%	More often a founding mutation	Loss of function	No impact on prognosis [25,100]
IDH1/2	2–5%	Typical subclonal mutation	Neomorphic enzyme	Leukemic progression Inferior survival [4]
Chromatin modification				
ASXL1	15–35%	More often a founding mutation	Loss of function	Leukemic progression Inferior survival Resistance to ruxolitinib [4,6,31,101]
EZH2	3–8%	More often a founding mutation	Loss of function	Leukemic progression Inferior survival [4,25,34]
Transcription factors				
RUNX1	2–5%	Typical subclonal mutation	Loss of function	Leukemic progression Inferior survival [25,102]
NFE2	2–5%	Typical subclonal mutation	Enhanced wild-type protein function	Conflicting results on prognosis [103–104]
RNA splicing				
SF3B1	2–5%	More often a founding mutation	Loss of function	No impact on prognosis Presence of ringed sideroblasts in bone marrow [25,105]
SRSF2	10–20%	More often a founding mutation	Loss of function	Leukemic progression Inferior survival [4,27]
U2AF1	5–10%	More often a founding mutation	Loss of function	Cytopenias Q157 variant associated with inferior survival [36,61]
ZRSR2	5–10%	More often a founding mutation	Loss of function	No impact on prognosis [25]
DNA repair control				
TP53	1–3%	Typical subclonal mutation	Loss of function	Leukemic progression Inferior survival [25]
PPM1D	0–2%	Typical subclonal mutation	Gain of function	Not defined [26]
Cell signaling				
SH2B3 (LNK)	2–5%	More often a founding mutation	Loss of function	Synergy with $JAK2^{V617F}$ mutationRole in familial cases [25,106]
CBL	2–5%			

(*continued on next page*)

Table 1 (*continued*)

Biological pathways and genes	Frequency	Mutation acquisition	Type of abnormality	Clinical correlations
		More often a founding mutation	Dominant negative	Leukemic progression Inferior survival Resistance to ruxolitinib [7,25]
NRAS	1–3%	Typical subclonal mutation	Gain of function	Leukemic progression Inferior survival Resistance to ruxolitinib [7,107]
KRAS	1–3%	Typical subclonal mutation	Gain of function	Leukemic progression Inferior survival Resistance to ruxolitinib [7,107]
PTPN11	1–3%	Typical subclonal mutation	Gain of function	Inferior survival in blast phase [25,102]

PMF, underpinning the worse prognosis of the latter [18].

Impact of molecular profiling on clinical features and prognosis

Clinico-hematological profile

JAK2-mutated MF patients have higher leukocyte counts than *MPL*- and *CALR*-mutated patients [16,17]. In turn, MF patients with *CALR* mutations are usually younger [15,16,32], with higher platelet counts and lower incidence of anemia and spliceosome mutations [32]. Studies have found no consistent differences in the clinical phenotype of *MPL*-mutated PMF compared to *JAK2*-mutated PMF [17,33]. In contrast, TN-MF patients are older and tend to have lower hemoglobin levels and platelet counts [17].

With regard to additional somatic mutations, *EZH2* mutations are correlated with higher leukocyte counts, blast cell counts, and a larger spleen size at PMF diagnosis [34]. *CBL/NRAS/KRAS* mutations are associated with poor clinical features, including higher leukocyte and blast cell counts, lower hemoglobin and platelet counts, and higher frequency of constitutional symptoms [7]. Patients with the myelodepletive phenotype of MF have low peripheral blood counts and often require blood transfusions [35]. They have limited treatment options, as use of JAK inhibitors or cytoreductive agents can exacerbate cytopenias. It has recently been shown that this phenotype is enriched with mutations in *U2AF1* gene in both PMF and SMF [36,37].

Thrombotic risk

Thrombosis is one of the leading causes of morbidity and mortality in MF patients [10]. In a Swedish population-based study, patients with MF across all age groups had a significantly increased rate of arterial and venous thrombosis compared with matched control participants, with the highest rates at or shortly after diagnosis [38]. In the largest series reported to date including 707 PMF patients, the overall cumulative rate of cardiovascular death and nonfatal thrombotic complications during follow-up was 7.2%, accounting for 1.75 events per 100 patient-years [39], figures in line with those of ET, a well-established thrombophilic condition. In a multivariable model, age older than 60 years and *JAK2*-mutated status were significantly associated with higher thrombosis rates. An updated analysis from the same group showed that fatal and non-fatal thrombotic events occurred in <1% patient-years in *CALR*- or *MPL*-mutated PMF patients, whereas no events were registered among triple-negative cases [40]. In contrast, the incidence of thrombosis in SMF was not different between the *JAK2* and *CALR* genotypes, although this observation did not take into account the potential effect of treatment [16]. Given the association between clonal hematopoiesis and arteriosclerotic cardiovascular disease [41], evaluating the impact of additional somatic mutations on thrombotic risk in MF is of clear interest.

Consensus recommendations for the prevention and management of thrombotic events in patients with MF are lacking. In clinical practice, treatment is individualized based on clinical judgment and extrapolation of data from studies in other MPNs, taking into account the higher risk of bleeding in MF patients. In general, patients with prefibrotic MF are treated according to ET guidelines due to their overlapping clinical features. The International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET) [42], a stratification model originally derived from ET patients that includes the $JAK2^{V617F}$ mutation as a risk factor, has recently been shown to accurately discriminate risk groups in prefibrotic PMF patients [43].

Risk of leukemic transformation

MF has an intrinsic propensity to evolve into AML, with an incidence of 10–20% in the first 10 years after diagnosis [44], and this complication is the main cause of death [10]. Unfortunately, no therapeutic agent has proven effective in preventing or delaying acute transformation in MF. The spectrum of molecular abnormalities observed in AML arising from MF is different to that of *de novo* AML [45]. Therefore, somatic mutations that are frequently detected in *de novo* AML, such as those affecting *FLT3*, *NPM1* and *DNMT3A* genes, are rare in post-MF AML. By contrast, mutations in genes of epigenetic regulators (*ASXL1*, *TET2*, *EZH2*, *IDH1/2*) and spliceosome components (*SRSF2*, *U2AF1*) are enriched in blast-phase MF. Of note, most of these mutations can already be detected in the chronic phase of the disease, while some of them, such as those in *TP53*, *RAS* or *RUNX1* genes can occasionally be detected from diagnosis at low levels but preferentially accumulate at the time of leukemic transformation [25,46–48].

Molecular studies performed in paired samples from MPN patients in the chronic and acute phases have demonstrated that a proportion of $JAK2^{V617F}$ MF cases can transform to AML from a *JAK2*-unmutated clone [47–50]. Among the different genotypes, TN-MF patients seem to have the highest risk of AML, whereas *CALR*-mutated patients have the lowest risk [16,17,47]. Nevertheless, the risk of acute transformation is modulated by the spectrum of additional somatic mutations present in each MF patient. In this respect, mutations affecting *ASXL1, SRSF2*, *IDH1/2*, *EZH2*, *U2AF1, RAS*, and *TP53* genes have been recurrently associated with a higher incidence of AML in MF patients [4,7,31,34]. Moreover, the presence of two or more high risk mutations was linked to shortened leukemia-free survival in an international study of 797 PMF patients [51].

Recently, the Mayo Clinic group [52] developed a predictive model for leukemic transformation in a series of 1306 PMF patients. Based on clinical variables (age > 70 years, circulating blasts ≥ 3%, sex-adjusted anemia) and molecular data (mutations in *IDH1*, *ASXL1* and *SRSF2*), the model can discriminate three risk categories with an estimated leukemic incidence of 57%, 17% and 8%, respectively. From a practical standpoint, this scoring system provides useful prognostic information to assist treatment decision making, especially with respect to candidate selection and timing of allo-HCT.

Impact of gene mutations on survival

Although the median survival of patients with MF is 6 years [10], there is great individual variability, with some patients surviving less than two years, compared with others still alive >20 years after diagnosis [53]. Given this heterogeneity, risk stratification is crucial for an individualized treatment approach, particularly regarding candidate selection for allo-HCT among younger MF patients [54], and inclusion

criteria for clinical trials.

Published data support that *CALR*-mutated PMF and SMF patients have superior survival to those with other genotypes, whereas TN-MF patients have the worst outcomes [16,17,32]. In PMF, the survival advantage of *CALR* mutations seems to be restricted to patients carrying the more common type 1/type 1-like mutations [24,55], although this association has not been confirmed in all series [22]. Among JAK^{V617F}-mutated PMF patients, those with a low V617F allele burden at diagnosis had shortened survival [56–58]. Several studies have demonstrated the detrimental effect of somatic mutations in *ASXL1*,*IDH1*, *IDH2*, *SRSF2*, *EZH2*, *U2AF1*, *TP53*, *NRAS*, and *KRAS* genes on survival of MF patients [4,7,14,34]. Accordingly, patients harboring one or more of these mutations are usually considered to have high molecular risk (HMR). Whether mutations are clonal or subclonal has shown little effect on prognosis [26]. While the frequency of HMR mutations is comparable in PMF and SMF, they have shown less impact on survival in SMF, with the single exception of mutations in *SRSF2* gene in post-ET MF [27,31].

The abovementioned molecular data have been included in several prognostic scoring systems that are useful tools to predict the outcome of MF patients managed with conventional drug therapies [26,59–62], as shown in Table 2. In general, these models can discriminate different risk groups for overall survival and leukemia-free survival. The main differences between them regard the target patient population (PMF, SMF or all MF subtypes), inclusion or not of cytogenetic abnormalities as a risk factor, and minor variations in HMR categorization. The Grinfeld et al model differs in that rather than discriminating risk groups, it makes an individual prediction of survival and leukemic risk based on a broad set of clinical characteristics, cytogenetics, and comprehensive molecular data.

Impact of molecular profiling on transplant decision making

Despite improvements in MF management over the years, allo-HCT remains the only curative therapy. However, its significant morbidity and mortality requires a very careful candidate selection from among fit patients with high-risk disease. In clinical practice, high risk patients are conventionally defined as those with an expected survival of under 5 years, as determined by the available prognostic scoring systems [54]. In this respect, contemporary stratification models that include cytogenetic and molecular risk factors [26,59,61,62] have led to more comprehensive prognostication [63].

Nonetheless, assessment of the risk–benefit balance of the procedure requires estimating the potential outcome of allo-HCT in each MF patient, for which several variables such as patient age, Karnofsky performance status (KPS), comorbidities or donor type are established risk factors for transplantation outcome [54]. Currently, clinicians can also apply the Myelofibrosis Transplant Scoring System (MTSS) to estimate survival in MF patients undergoing allo-HCT [64]. By integrating patient-, disease- and transplant-specific factors (Table 2), this latter model defines four risk categories, with a 5-year post-transplant survival of 83%, 64%, 37%, and 22% for low, intermediate, high, and very high risk groups, respectively.

In conclusion, adding molecular data to current prognostic tools has enabled the greater discriminative accuracy essential for transplant decision making in MF.

Impact of molecular profiling on medical treatment

In clinical practice, most patients with MF (~90%) are not eligible for transplantation and their treatment will be directed to symptomatic control and prevention of disease complications [65]. Available pharmacological therapies can essentially be grouped into (a) treatments aimed at improving anemia (erythropoiesis stimulating agents, danazol, prednisone, immunomodulatory agents); and (b) agents to control the hyperproliferative manifestations of MF (cytoreductive agents, JAK inhibitors). Data is lacking on the influence of molecular abnormalities on

Table 2
Prognostic scoring systems for survival in myelofibrosis which include molecular data.

Prognostic model	Clinical factors (Score points)	Genetic factors (Score points)	Risk groups (Median or 5-yr OS)
MYSEC-PM [60]	Hb < 11 g/dL [2] Blood blasts ≥ 3% [2] Platelets < 150 × 10^9/L [1] Constitutional symptoms [2] Age (0.15/yr)	Non-*CALR* [2]	Low (not reached) Intermediate-1 (9.3 yr) Intermediate-2 (4.4 yr) High (2.0 yr)
MIPSS70 [59]	Hb < 10 g/dL [1] Blood blasts ≥ 2% [1] Constitutional symptoms [1] Bone marrow fibrosis ≥ 2 [1] WBC > 25 × 10^9/L [2] Platelets < 100 × 10^9/L [2]	Non-*CALR* type-1 [1] HMR[a] = 1 [1] HMR[a] ≥ 2 [2]	Low (27.7 yr) Intermediate (7.1 yr) High (2.3 yr)
GIPSS [62]	None	Non-*CALR* type-1 [1] *ASXL1* [1] *SRSF2* [1] $U2AF1^{Q157}$ [1] HR karyotype[b] [1] VHR karyotype[b] [2]	Low (26.4 yr) Intermediate-1 (8.0 yr) Intermediate-2 (4.2 yr) High (2.0 yr)
MIPSS70 + v2.0 [61]	Moderate anemia[c] [1] Severe anemia[d] [2] Blood blasts ≥ 2% [1] Constitutional symptoms [2]	Non-*CALR* type-1 [2] HMR[e] = 1 [2] HMR[e] ≥ 2 [3] HR karyotype[b] [3] VHR karyotype[b] [4]	Very low (not reached) Low (16.4 yr) Intermediate (7.7 yr) High (4.1 yr) Very high (1.8)
MPN Personalized Risk Calculator [26]	Age/Sex Hb/WBC/Platelets Prior thrombosis Splenomegaly	Mutations in 33 genes Cytogenetic abnormalities	Individualized risk calculator
MTSS [64]	Platelets < 150 × 10^9/L [1] WBC > 25 × 10^9/L [2] KPS < 90% [1] Age ≥ 57 years [1] Mismatched UNR donor [2]	Non *CALR/MPL* [2] *ASXL1* [1]	Low (83%) Intermediate (64%) High (37%) Very high (22%)

OS, overall survival; Hb, hemoglobin; WBC, leukocytes; KPS, Karnofsky performance status; UNR, unrelated.
MYSEC-PM: Myelofibrosis Secondary to PV and ET-Prognostic Model. On-line calculator: www.mysec-pm.eu.
MIPSS70: Mutation-Enhanced International Prognostic Scoring System. On-line calculator: www.mipss70score.it.
GIPSS: genetically inspired prognostic scoring system.
MIPSS70 + v2.0: Mutation-Enhanced International Prognostic Scoring System plus version 2.0. On-line calculator: www.mipss70score.it.
MTSS: Myelofibrosis Transplant Scoring System.
MPN Personalized Risk Calculator. On-line calculator: htpps://www.sanger.ac.uk/science/mpn-multistage/.
[a] High molecular risk (HMR) mutations in *ASXL1*, *SRSF2*, *EZH2*, or *IDH1/2* genes.
[b] Very high risk (VHR): single/multiple abnormalities of −7, i(17q), inv [3]/3q21, 12p−, 11q−/11q23, +21, or other autosomal trisomies, not including +8/+9. Favorable: normal karyotype or sole abnormalities of 20q-, 13q-, +9, t/dup chrom 1, or sex chromosome abnormality including -Y. Unfavorable or high risk (HR): all other abnormalities.
[c] Hb 8–9.9 g/dL in women or Hb 9–10.9 g/dL in men.
[d] Hb < 8 g/dL in women or Hb < 9 g/dL in men.
[e] High molecular risk (HMR) mutations in *ASXL1*, *SRSF2*, *EZH2*, *IDH1/2* or $U2AF1^{Q157}$ genes.

medical treatment outcomes in MF patients, but in the following sections we summarize what has been published in this topic.

Erythropoiesis stimulating agents (ESA)

These agents constitute the treatment of choice in case of anemia with inadequate serum erythropoietin levels [in practice, <125–150 U/L] [65]. Anemia response is observed in approximately 40% of patients treated with ESA, with a median response duration of around 12 months [66]. Elevated serum ferritin levels at baseline and red blood cell transfusion dependence are associated with a lower probability of response [67,68], whereas driver mutation subtype does not seem to influence results [68,69].

Interferon (IFN)

INF-α has immunomodulatory and anti-proliferative effects on hematopoietic progenitors. Historically, use of IFN has been limited in MF due to the high incidence of side effects. More recently, improved tolerability of the pegylated forms of IFN-α has resulted in more favorable results, although mainly restricted to MF patients with hyperproliferative phenotype and moderate splenomegaly [70]. In a French series of 62 MF patients treated with pegylated IFN, the *JAK2*^{V617F} allele burden decreased by >50% in a significant proportion of patients (60%), but molecular responses did not correlate with the main outcomes [71]. In this study, *CALR*-mutated patients had longer median survival than *JAK2*-mutated ones (13.5 vs. 7 years, respectively), whereas the presence of at least one additional somatic mutation was associated with reduced overall survival and leukemia-free survival. In another retrospective study, MPN driver mutation type did not correlate with response to IFN-α, whereas the presence of HMR abnormalities negatively affected treatment response [72].

Immunomodulatory drugs (IMiDs)

Immunomodulatory drugs (lenalidomide, thalidomide), usually combined with low-dose prednisone during the first 3 months, can improve anemia in a quarter of MF patients and raise platelet counts in some of them, but are usually ineffective in controlling splenomegaly [65]. In a retrospective study including 176 consecutive MF patients who received lenalidomide or thalidomide for at least four weeks, splicing mutations (such as *SRSF2* or *U2AF1*Q157 mutations) were frequently detected, but lacked prognostic implications regarding treatment response [73].

Ruxolitinib

The most effective strategy to control the hyperproliferative manifestations of MF is by pharmacological inhibition of JAK kinases. In the pivotal COMFORT studies, the use of ruxolitinib, a JAK1 and JAK2 inhibitor, was associated with a significant improvement in symptomatic burden in most patients, while about 50% had decrease in spleen volume of at least 35% [50% size reduction by palpation] [74]. Primary resistance was rare and suboptimal response (loss of clinical benefit) developed more frequently in the setting of dose adjustment for hematologic toxicity. Overall, the median duration of spleen responses was 3 years. Ruxolitinib does not have the capacity to eradicate the neoplastic clone of MF, since neither allelic load of the mutated form of *JAK2* nor medullary fibrosis is substantially reduced in most patients [75,76]. Thus, the average reduction in *JAK2* allelic burden was 7–22% after 48 weeks of treatment in evaluable patients [77,78], whereas regression of bone marrow fibrosis was seen in around 16% of patients at last follow-up [79]. Interestingly, in a series of 236 *JAK2*^{V617F}-mutated patients from the COMFORT-1 trial, greater allele burden decreases correlated with changes in spleen volume but not with other clinical/hematologic parameters, bone marrow morphology or constitutional symptoms [75].

The type of MPN driver mutation does not seem to correlate with spleen response or hematologic toxicity with ruxolitinib [80,81]. In contrast to findings in other target-directed therapies, ruxolitinib resistance is not associated with occurrence of *JAK2* catalytic domain mutations in MF patients, which could indicate either incomplete inhibition of JAK2 or that MF is not critically dependent on JAK2 kinase activity [76]. Instead, it has been observed experimentally that cells from MF patients with prolonged exposure to ruxolitinib can develop treatment resistance by reactivation of the JAK-STAT pathway through heterodimerization of the activated form of JAK2 with JAK1 or TYK2 [82]. In addition, the presence of one or more HMR mutations at baseline or acquired during treatment have been associated with increased risk of ruxolitinib resistance [5,6,81].

Novel therapeutic agents

Molecular profiling is used in clinical practice to identify potential treatment targets in patients with different malignancies, in an approach termed precision medicine. Until now, MPN driver mutation type has shown no significant influence on the results of available medical treatments in MF patients, but it could have a role in directing future therapeutic strategies. In this sense, the valine-to-phenylalanine mutation in *JAK2* gene and the novel C-terminus of *CALR* mutations represent neoantigens that could generate a vaccine-specific immune response [83,84], and clinical trials exploring this approach are ongoing. *IDH1/2* mutations are detected in only 2–5% of MF patients in the chronic phase, but around 20% in the AML phase [85], offering the opportunity of IDH-inhibitor based therapy with ivosidenib or enasidenib. In a retrospective study including 12 *IDH1/2*-mutated patients with post-MPN AML treated with IDH1/2 inhibitors in monotherapy or in combination with other treatments, three of them achieved complete remission with undetectable *IDH1/2* mutations by NGS [86]. Median survival of the series was 10 months, which compares favorably with historically modest survival in post-MPN AML [87]. Finally, molecular profiling might help in predicting response to novel compounds. In this regard, the complete response rate with the telomerase inhibitor imetelstat was better in MF patients harboring *SF3B1* or *U2AF1* mutations than among those without mutations in these genes [38% vs. 4%] [88].

Table 3
Impact of somatic mutations on survival after allogeneic hematopoietic cell transplantation.

Reference	No. of patients	Institution	*JAK2* mutation	*CALR* mutation	*ASXL1* mutation
Kroger et al., 2017 [89]	169	Germany	NE	Good	Adverse
Samuelson et al., 2018 [108]	233	Seattle	None	NE	NE
Ali et al., 2019 [91]	110	City of Hope	None	None	None
Tamari et al., 2019 [92]	101	US-Canada	None	None	None
Gagelmann et al., 2019 [64]	361	Germany/ France	Adverse	Good	Adverse
Hernandez-Boluda et al., 2020 [90]	197	Spain	None	None	None
Lwin et al., 2020 [109]	142	Australia	None	NE	NE
Hernandez-Boluda et al., 2021 [13]	588	EBMT	NE	Good	NE

NE: not evaluated.

Impact of molecular profiling on transplantation

Conflicting results have been published regarding the impact of molecular abnormalities on transplantation outcomes (Table 3). In some European series, *CALR*-mutated patients had better survival than those with other genotypes [13,64,89], and *ASXL1*-mutated patients had higher relapse incidence [64,89], but these findings have not been confirmed in other studies [90–92] In a series of 95 patients with post-MPN AML, detection of *TP53* gene mutations by NGS at the time of allo-HCT was associated with a higher risk of relapse and worse survival, suggesting that the adverse impact of these mutations could not be overcome by transplantation [93].

MPN driver mutations are present in most MF patients and can therefore be useful to monitor minimal residual disease post-transplantation. Definition of molecular persistence or relapse after allo-HCT in MF patients is complicated due to the variable clearance kinetics of detectable mutations [94]). However, persistent detection of MPN driver mutations by quantitative high-sensitivity assays has been associated with higher risk of relapse and worse survival in several studies [95,96]. In a series of 136 MF patients from Hamburg, detectable molecular disease at day +100 or at day +180 was associated with higher risk of clinical relapse within 5 years compared to patients with undetectable disease [62% vs. 10% and 70% vs. 10%, respectively] [96]. Consequently, molecular monitoring may be useful to make timely therapeutic decisions to prevent relapse, such as tapering of immunosuppressive drugs or infusion of donor lymphocytes. In this respect, the Hamburg group has also shown that complete molecular remission following donor lymphocyte infusions can be more frequently achieved when triggered by persistence of molecular disease than in frank clinical relapse [100% vs. 44%] [97].

Finally, screening for additional somatic mutations by NGS could potentially be used for disease monitoring after allo-HCT, but data on this strategy in MF is still scarce. In a recent study [98], Mannina et al. demonstrated the feasibility of digital-droplet PCR for monitoring residual disease after allo-HCT in MF patients with *IDH1*, *IDH2* and *DNMT3A* mutations. This approach could be particularly useful for molecular monitoring in TN-MF patients.

Conclusions

Accumulating data on molecular profiling is bringing new insights into the pathogenic mechanisms involved in the highly heterogeneous clinical course of MF. MPN driver mutations in *JAK2*, *CALR* and *MPL* genes are detected in most MF patients and underpin the constitutive activation of the JAK-STAT pathway leading to myeloproliferation. The four major MF genotypes (*JAK2*, *CALR*, *MPL*, triple negative) display differences in presenting clinical features and main outcomes. NGS panel testing has shown that more than half of MF patients have somatic mutations in addition to the MPN drivers already detectable at time of diagnosis, and these may accumulate along the disease course. These mutations are not specific to MPNs but are known to contribute to the pathogenesis of other myeloid neoplasms, and some are considered high molecular risk (HMR) mutations due to their association with adverse outcomes, such as higher risk of leukemia and shorter overall survival.

In clinical practice, the main role of molecular profiling is prognostication, principally for selection of high-risk patients who may be candidates for transplantation, the only curative treatment for MF to date. To this end, contemporary prognostic models with incorporated molecular data are useful tools to discriminate different risk categories. Patient stratification by disease risk group is also being used for selection criteria in clinical trials to define more homogeneous patient populations. With the exception of a few clinical situations such as thrombosis prevention, decisions on medical treatment are not based on patient molecular characterization due to the limited impact of somatic mutations on outcomes using conventional agents. Nonetheless, molecular data may become more important in novel treatment strategies, such as the use of immunotherapy with vaccines against the mutant forms of JAK2 or CALR, or the use of drugs directed against actionable molecular targets, such as mutant IDH1/2 variants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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