Current Concepts of Pathogenesis and Treatment of Philadelphia Chromosome-Negative Myeloproliferative Neoplasms

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Hamostaseologie 2021;41:197–205.

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Abstract

Philadelphia chromosome-negative myeloproliferative neoplasms are hematopoietic stem cell disorders characterized by dysregulated proliferation of mature myeloid blood cells. They can present as polycythemia vera, essential thrombocythemia, or myelofibrosis and are characterized by constitutive activation of *JAK2* signaling. They share a propensity for thrombo-hemorrhagic complications and the risk of progression to acute myeloid leukemia. Attention has also been drawn to *JAK2* mutant clonal hematopoiesis of indeterminate potential as a possible precursor state of MPN. Insight into the pathogenesis as well as options for the treatment of MPN has increased in the last years thanks to modern sequencing technologies and functional studies. Mutational analysis provides information on the oncogenic driver mutations in *JAK2*, *CALR*, or *MPL* in the majority of MPN patients. In addition, molecular markers enable more detailed prognostication and provide guidance for therapeutic decisions. While *JAK2* inhibitors represent a standard of care for MF and resistant/refractory PV, allogeneic hematopoietic stem cell transplantation remains the only therapy with a curative potential in MPN so far but is reserved to a subset of patients. Thus, novel concepts for therapy are an important need, particularly in MF. Novel *JAK2* inhibitors, combination therapy approaches with ruxolitinib, as well as therapeutic approaches addressing new molecular targets are in development. Current standards and recent advantages are discussed in this review.

Keywords

- myeloproliferative neoplasm
- JAK2 signaling
- pathophysiology
- management

Introduction

Myeloproliferative neoplasms (MPNs) are hematopoietic stem cell disorders that comprise the Philadelphia-positive chronic myeloid leukemia as well as Philadelphia-negative entities. This review focuses on polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF) that belong to the Philadelphia-negative MPNs and will be referred to as MPNs in this review. Other Philadelphia-negative forms such as chronic neutrophilic leukemia, chronic eosinophilic leukemia, and MPN-unclassified are reviewed elsewhere.

Primary myelofibrosis (PMF) develops de novo, but MF can also progress from preexisting PV or ET, then referred to as post-PV or post-ET myelofibrosis (PPV-/PET MF).[1] These MPN subtypes present with characteristic phenotypes: PV with erythrocytosis potentially accompanied by thrombo-/leukocytosis, ET with thrombocytosis, and PMF with bone marrow fibrosis and subsequent cytopenia. They share a propensity for thromboembolic events and bleeding,

received
February 4, 2021
accepted after revision
March 17, 2021

Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI https://doi.org/10.1055/a-1447-6667.
ISSN 0720-9355.

development of bone marrow fibrosis, and transformation to acute myeloid leukemia (AML). On the molecular level, all three MPN subtypes are characterized by activation of Janus kinase 2 *(JAK2)* signaling.[2]

Despite the rapidly increasing knowledge regarding pathophysiology and treatment options, there are still unmet clinical needs, especially in MF. Treatment response is lost in 50% of patients after 5 years and survival is limited after stopping therapy.[3] Hence, new therapeutic strategies are urgently needed.

Molecular Pathogenesis of Myeloproliferative Neoplasms

The majority of MPNs harbor a mutation in the pseudokinase domain of *JAK2*.[4] In a physiologic setting, *JAK2* is essential for the proliferation of hematopoietic cells. Ligand binding to hematopoietic cytokine receptors such as erythropoietin (*EPOR*), thrombopoietin (*TPOR* or *MPL*), and GM-CSF receptors induces dimerization and transphosphorylation of the receptor and activation of *JAK2*, which mediates hematopoietic cytokine signaling. *JAK2* activates several signaling pathways promoting proliferation, differentiation, and survival including the signal transducer and activator of transcription (*STAT3/5*) pathway, the mitogen-activated protein kinase (*MAPK*) and the phosphoinositide 3-kinase (*PI3K*) signaling pathways.[5] In MPNs, *JAK2* signaling is constitutively activated by somatic mutations in *JAK2*, calreticulin (*CALR*), or the thrombopoietin receptor *MPL* leading to dysregulated cell proliferation (►Fig. 1). These "phenotypic driver mutations" are sufficient to induce MPN features and occur largely mutually exclusive.[6] Rare cases of *JAK2* mutant MPN with a concomitant second driver mutation in *MPL* or *CALR* have been reported.[7,8]

The *JAK2 V617F* mutation was first described in 2005 as a gain-of-function point mutation.[9–12] *JAK2 V617F* is found in 95% of PV patients and 50 to 60% of ET and MF patients. In PV, mutations in exon 12 of *JAK2* may also occur at a lower frequency and are associated, although not in all cases, with isolated erythrocytosis and a younger age at diagnosis.[13] *MPL* mutations are found in 5 to 10% of ET and MF and are absent in PV.[14,15] In the chaperone protein *CALR*, more than 35 mutations have been identified, which are located in exon 9 of *CALR* and most frequently lead to a 52 bp deletion, referred to as type 1 mutation, or a 5 bp insertion, referred to as type 2 mutation.[16,17] It has been shown that *CALR* mutations result in the activation of *MPL* thereby inducing *JAK2* signaling.[18] Patients with *CALR* mutant ET are often diagnosed at younger age and have a more favorable prognosis as compared with *JAK2* or *MPL* mutant MPN, particularly in the case of type 1

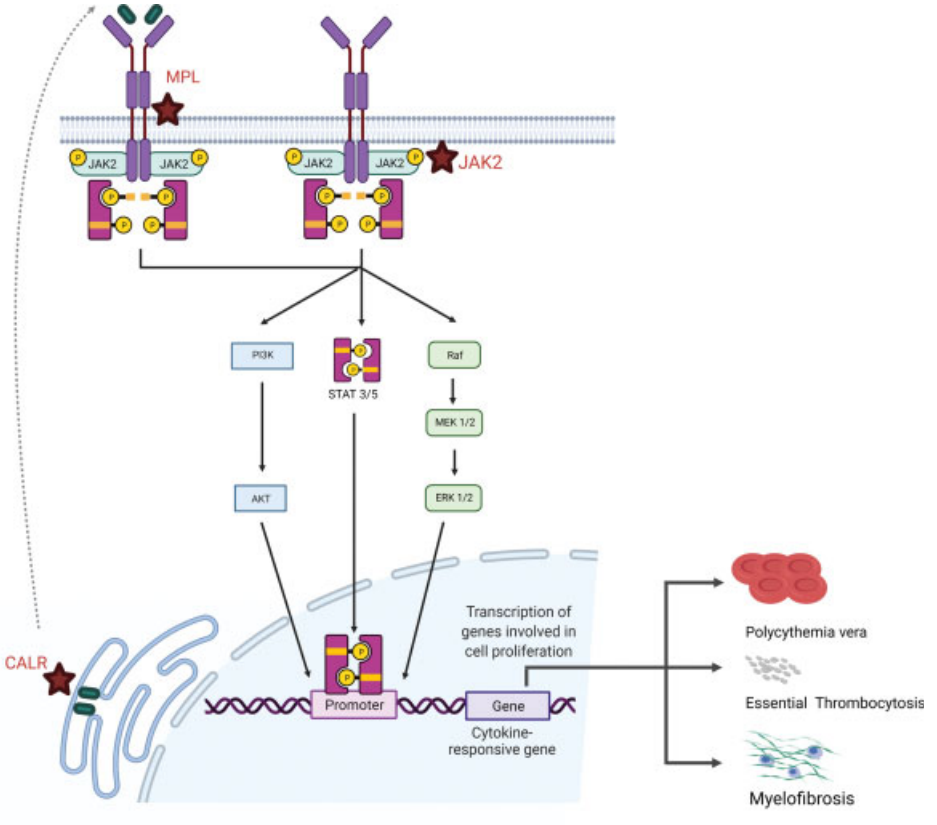

Fig 1 Main pathophysiologic mechanisms in myeloproliferative neoplasms (MPNs). MPNs are characterized by constitutive activation of JAK2 signaling promoted by somatic mutations in JAK2, CALR, or MPL (indicated with stars). Activation leads to dimerization and transphosphorylation of the receptor. Consequently, STAT-, MAPK-, or PI3K signaling is activated leading to transcription of genes causing cell proliferation and survival. Created with BioRender.com.

CALR mutations.[16,17] In approximately 10% of ET and MF patients referred to as *triple negative* MPN, no mutations in *JAK2*, *CALR*, or *MPL* are detected.[19] Triple negativity negatively impacts on prognosis in PMF, while triple negative ET shows mixed pathogenesis with atypical mutations in *JAK2* or *MPL*, very low allele burden of typical mutations, but also inherited mutations.[20]

Additional gene mutation, known to be prevalent also in other myeloid malignancies such as myelodysplastic syndrome and AML, are frequently detected in MPNs, particularly MF. Most common are mutations in *TET2*, *DNMT3A*, *ASXL1*, *EZH2*, *TP53*, *IDH1*, *IDH2*, *SRSF2*, *NF1*, and *NRAS*.[17,21,22] The presence of the so-called high-molecular-risk (HMR) mutations including *ASXL1*, EZH2, IDH1, IDH2, and *SRSF2*, as well as the number of cooccurring nondriver mutations are associated with a worse prognosis.[21–23] Patients harboring mutations in *TP53* are particularly at risk for transformation to AML and also *IDH1/2* mutations are enriched in post-MPN AML.[22,24]

Clinical Presentation of Myeloproliferative Neoplasms

MPNs typically present with cytoses due to the excessive production of mature myeloid blood cells.[1] Erythrocytosis in PV and thrombocytosis in ET, MF, and potentially PV induce rheologic alterations, which can contribute to headaches, impaired vision, or paresthesia. Aquagenic pruritus is a typical feature in PV. Platelets circulating in an activated state further increase the risk of thromboembolic events both in arterial and venous vascular beds. Thromboembolic complications associate particularly with PV and ET but may also occur in MF. Thrombosis in atypical sites such as splanchnic thrombosis, Budd–Chiari syndrome, or cerebral sinus vein thrombosis is characteristic and should trigger the evaluation for an underlying MPN. The risk of bleeding is increased in all three subtypes of MPNs. It relates to extreme thrombocytosis (>1,500 G/L), which leads to acquired von Willebrand syndrome via depletion of unusually large von Willebrand factor multimers, or to thrombocytopenia in MF.

Splenomegaly due to extramedullary hematopoiesis is frequent in MPNs and most pronounced in MF. It can cause abdominal discomfort and early satiety and require therapeutic intervention. Furthermore, MPN patients typically suffer from constitutional symptoms including night sweats, fever, weight loss, and fatigue.[1] They relate to an inflammatory milieu with increased cytokine levels due to activated *JAK2* signaling. MF patients present with the highest symptom burden. The Myeloproliferative Neoplasm Symptom Assessment Form Total Symptom Score is a useful tool for standardized monitoring of symptom burden.[25] MF can present as a prefibrotic form with minimal fibrosis, thrombocytosis, and/or leukocytosis or as overt MF with substantial bone marrow fibrosis and progressive cytopenia.[26,27] Progressive bone marrow failure leads to symptomatic anemia and increased risk for infection and bleeding. Transformation to AML occurs in 10 to 20% of MF patients and is associated with a poor prognosis.[24]

Diagnosis of Myeloproliferative Neoplasms

The suspicion of MPN should be raised upon persistent erythrocytosis, thrombocytosis, or neutrophilia, particularly when causes for reactive cytoses are improbable.[26] Concomitant thromboembolic complications, particularly in atypical sites, make a diagnosis of MPN highly probable. Anemia and/or thrombocytopenia along with myeloid and erythroid precursors in the peripheral blood (leucoerythroblastic blood smear) and tear-drop–shaped red cells or progressive splenomegaly should trigger investigation for MF. Diagnosis is made according to the revised World Health Organization (WHO) criteria based on blood counts, bone marrow morphology, and the presence of a *JAK2*, *CALR*, or *MPL* mutation (►Table 1).[26] In triple negative MF, extended genetic analyses are suggested to investigate for mutations in *ASXL1*, *EZH2*, *TET2*, *IDH1*, *IDH2*, *SRSF2*, or *SF3B1* to demonstrate clonality. Typical findings of bone marrow morphology are central to the diagnosis of all three MPN subtypes. Bone marrow biopsy is dispensable for the diagnosis of PV if erythrocytosis is pronounced (hemoglobin 185 g/L/> 165 g/L or hematocrit 55.5%/49.5 in men/women, respectively). However, bone marrow examination is still recommended in these settings to assess bone marrow fibrosis, which is of prognostic relevance.

Diagnosis of ET, which relies on thrombocytosis, typical changes of megakaryopoiesis, and detection of an MPN-typical driver mutation, should be carefully evaluated versus the new entity of prefibrotic MF.[27] Blood, bone marrow, and molecular features are overlapping with ET and the differences in bone marrow morphology, which allow to distinguish ET from prefibrotic MF are subtle. However, proper identification of prefibrotic MF is meaningful, as prognosis is different from ET with a higher risk of progression to overt MF and shorter overall survival.

JAK2 Mutant Clonal Hematopoiesis of Indeterminate Potential

Mutations in *DNMT3A, TET2, ASXL1,* as well as other mutations frequently seen in myeloid malignancies have also been identified in healthy individuals with normal blood counts. This clonal hematopoiesis of indeterminate potential (CHIP) occurs at increasing frequency at higher age. It has been described as a clonal expansion of hematopoietic cells due to age-related somatic mutations without apparent hematologic malignancy.[28,29] CHIP is associated with an up to 14-fold risk to develop myeloid neoplasms or cardiovascular complications.[30,31] Of note, *JAK2 V617F* is known to occur relatively frequently as CHIP and induces, as in manifest MPNs, chronic inflammatory changes and an increased risk for thromboembolic events.[32] Particularly noticeable is a significantly increased risk of coronary artery disease in *JAK2 V617F* CHIP as compared with CHIP with *DNMT3A, TET2,* or *ASXL1* mutations.[31] *JAK2* mutant MPN can develop from CHIP over time, but currently this findings do not yet translate into clinical recommendations on how to monitor individuals with CHIP.[32]

Table 1 Diagnostic criteria according to WHO 2016 classification[26]

Polycythemia vera All three major *or* Major 1 + 2 and minor	Essential thrombocythemia All four major *or* Major 1–3 and minor	Primary myelofibrosis All three major and at least one minor
Major criteria		
1. Women: hemoglobin > 16.0 g/dL or hematocrit > 48% Men: hemoglobin > 16.5 g/dL or hematocrit > 49% *or* increased red blood cell mass	1. Platelet count $\geq 450 \times 10^9$/L	1. Megakaryocytic proliferation and atypia, with reticulin and/or collagen fibrosis ≥ grade 2
2. Bone marrow: hypercellularity, trilineage proliferation, pleomorphic mature megakaryocytes	2. Bone marrow: megakaryocyte proliferation with enlarged, mature megakaryocytes with hyperlobulated nuclei, no left shift	2. Not meeting WHO criteria for other myeloid neoplasms
3. *JAK2 V617F* or *JAK2* exon12 mutation	3. Not meeting WHO criteria for other myeloid neoplasms	3. *JAK2* or *CALR* or *MPL* mutation *or* presence of another clonal marker *or* absence of reactive BM reticulin fibrosis
	4. *JAK2* or *CALR* or *MPL* mutation	
Minor criteria		
- Subnormal serum erythropoietin level	- Another clonal marker present *or* - No evidence of reactive thrombocytosis	- Anemia not attributed to comorbid condition - Leucocyte $\geq 11 \times 10^9$/L - Palpable splenomegaly - LDH elevation - Leukoerythroblastosis

Therapy of Myeloproliferative Neoplasms

The aims of MPN therapy are prevention of thromboembolic and hemorrhagic complications, symptom alleviation, and prevention of transformation to fibrosis or AML. As PV and ET show an overall high risk of thrombohemorrhagic events, but an overall indolent course with a low rate of transformation to AML, the primary goal is reduction of vascular complications.[1] Allogeneic hematopoietic stem cell transplantation (HSCT), the only therapeutic approach with curative potential, is reserved for patients with higher risk MF. Given the high prevalence of vascular complications in MPNs, addressing cardiovascular risk factors such as smoking habits, dyslipidemia, arterial hypertension, obesity, and diabetes is important.[33]

Polycythemia Vera

Low-dose acetyl salicylic acid (ASS) significantly reduces vascular complications in PV and is recommended in all PV patients without contraindications to antiplatelet therapy such as previous bleeding events or acquired von Willebrand syndrome.[34] The question of how completely erythrocytosis needs to be corrected was addressed by the CYTO-PV trial showing clearly lower rates of death from cardiovascular cause or major thrombosis in patients with hematocrit less than 45% as compared with 45 to 50%. Hence, all PV patients should strictly be managed at hematocrit values less than 45%.[35] Furthermore, increasing age and a history of thrombosis are established risk factors for vascular events. Therefore, PV patients older than 60 years and/or with previous thrombosis are considered at high risk and should receive cytoreductive treatment according to the European Leukemia Net (ELN) recommendations.[33] There is growing evidence that leukocytosis could be a risk factor for thrombosis, but to date studies are conflicting.[36–38]

First-line cytoreductive treatment is usually performed by hydroxyurea (HU). Nevertheless, ELN recommendations advise for the preferential use of pegylated interferon-α rather than HU as first-line cytoreductive therapy in younger patients.[33] Upon intolerance or resistance to HU, pegylated interferon-α or the JAK2 inhibitor ruxolitinib should be used as second-line cytoreductive agents.[39]

Interferon-α is used for the treatment of PV since the 1980s. Pegylation resulted in better tolerability and increased half-life. The ability of interferon-α to reduce clone size and induce molecular remissions makes it a preferred choice, particularly in younger patients, but clone-reducing effects may take time to develop.[40] Recently, Ropeginterferon alfa-2b, a monopegylated isoform of interferon-α was approved in the European Union and Switzerland for first-line cytoreductive therapy of early PV based on the PROUD-PV and CONTINUATION-PV studies.[41] The reduced application frequency of once every 2 weeks may help improve tolerability and adherence. As second-line cytoreductive agents, the *JAK1/2* inhibitor ruxolitinib represents a valid option. The RESPONSE study showed effective correction of erythrocytosis, splenomegaly and constitutional symptoms in patients intolerant or resistant to HU, who were treated with ruxolitinib.[42] Also in patients without splenomegaly, ruxolitinib proved to be effective.[43]

Essential Thrombocythemia

In ET, antiplatelet therapy with low-dose ASS is recommended by the ELN guidelines analogous to PV.[33] Studies on the potential of twice-daily ASS for improved prophylactic effects are ongoing.[44] For patients with low-risk ET (<60 years of age without history of thrombosis) positive for a CALR mutation, there is cumulative evidence that they show higher rates of bleeding and should not be exposed to ASS prophylaxis.[45] In patients with platelets greater than 1,000 G/L, acquired von Willebrand syndrome should be ruled out before starting ASS.[33]

The risk for thrombosis in ET has been assessed by the International Prognostic Score of Thrombosis for ET (IPSET-thrombosis). Beyond the classical factors (age and history of thrombosis), this score takes into account the presence of the *JAK2 V617F* mutation and cardiovascular risk factors stratifying patients into low, intermediate, and high risk.[46,47] Leukocytosis greater than 11 G/L seems to be an additional risk factor for death and potentially thrombosis.[48–50]

In ET patients older than 60 years, with a history of a thromboembolic event or a platelet count greater than 1,500 G/L, cytoreductive therapy should be initiated for further risk reduction.

HU and pegylated interferon-α are established as first-line therapies.[33] Conflicting data exist concerning the use of anagrelide. The PT1 study showed more frequent thromboembolic events and bleeding as compared with HU,[51] whereas the ANAHYDRET study found noninferiority of anagrelide compared with HU.[52] Generally, anagrelide should be avoided in patients with cardiovascular risk factors. Recently, an extended release formulation of anagrelide was developed that could offer a more convenient dosing schedule.[53] Ruxolitinib has been tested in ET intolerant or resistant to HU, but so far has not proven beneficial.[54,55] The ongoing RUXO-BEAT trial evaluates the role of ruxolitinib as first-line therapy in high-risk PV and ET, and results are awaited.

Myelofibrosis

Therapy of MF includes symptom-directed supportive measures, disease-modifying treatments, and allogeneic HSCT.[56] In contrast to PV and ET, risk stratification is not primarily focused on thrombohemorrhagic risk, but on overall outcome. Prognostication schemes have been continuously refined given the recent progress of insight into molecular factors in MF. Particularly, mutations with prognostic significance have been added to current stratification schemes and improve prognostication.[17,22] While the dynamic international scoring systems (DIPSS and DIPSS-plus) rely on clinical factors, blood counts, and cytogenetics, the more recent mutation-enhanced international prognostic scoring system for transplant-age patients (MIPSS-70 and MIPSS-70 plus) and genetically inspired prognostic scoring system (GIPSS) take into account a patient's mutational profile (►Table 2).[57–61]

In patients with low or intermediate-I risk MF, management is primarily symptom-oriented.[33] MF-associated anemia can be treated with erythropoietin-stimulating agents if endogenous erythropoietin is low (<125 IU/L). Androgens such as danazol may also be used except upon prostate or liver disease.[62] Immunomodulators such as pomalidomide with or without steroids represent an additional approach to counteract anemia without concomitant polyneuropathy. Patients not responding or not eligible to one of those therapies should be supported by red blood cell transfusions.[33]

Symptomatic splenomegaly, which is a frequent problem in MF, generally responds well to *JAK1/2* inhibitor treatment (e.g., by ruxolitinib), which is approved for intermediate- and high-risk patients based on the COMFORT trials.[63–65] HU represents an alternative option with response in approximately 40% of patients.[66] Splenic irradiation or splenectomy is increasingly infrequently used. Particularly splenectomy should be reserved for refractory patients given a high perioperative risk. *JAK1/2* inhibition with ruxolitinib is effective in reducing constitutional symptoms, an effect which may relate particularly to the *JAK1* inhibition positively impacting on the inflammatory milieu in MF.[67] As ruxolitinib is inhibiting not only mutated but also unmutated *JAK2*, benefits can be expected regardless of the *JAK2* mutational status and *CALR* or *MPL* mutant as well as triple negative MF patients also represent candidates for ruxolitinib therapy.[68]

Although ruxolitinib is generally well tolerated, anemia and thrombocytopenia are frequent given that normal hematopoiesis is inhibited. Anemia can be treated with supportive measures. Furthermore, varicella zoster virus reactivation is observed more frequently in patients treated with ruxolitinib. There are data raising concern regarding higher incidences of malignant B-cell lymphoma in patients receiving ruxolitinib; however, only patients with preexistent B-cell clone were affected.[69] Those patients should be regularly screened for lymphoma. Attention must be paid to a possible withdrawal reaction including respiratory distress syndrome upon abrupt discontinuation of the drug. The risk of severe withdrawal syndrome can be reduced but not completely avoided by tapering ruxolitinib.[70] However, specific guidance on tapering regimens is currently lacking. The concurrent use of corticosteroids and/or HU might be beneficial.[71]

Allogeneic HSCT represents the only curative therapeutic approach in MF to date and is currently recommended by the ELN and the European Society for Blood and Marrow Transplantation for patients with high-risk or intermediate-2-risk MF.[72] In intermediate-1-risk patients, evaluation of allogeneic HSCT is advised if additional adverse factors are present including transfusion dependence, poor-risk cytogenetics, triple negativity for driver mutations, or the presence of an *ASXL1* mutation. Thanks to the introduction of reduced intensity conditioning regimes and the continued improvement of supportive measures, allogeneic HSCT has become increasingly available for elderly patients.[72]

Given the substantial symptom burden and limited overall survival of higher risk forms of MF, patients with MF are in urgent need for improved therapeutic options. Additional *JAK2* inhibitors, combination therapies with ruxolitinib, as well as therapeutic options addressing new targets are

Table 2 Prognostic scores in myelofibrosis

	DIPPS[57]	DIPSS-plus[58]		MIPSS70[59]		MIPSS70+ version 2.0[60]	
Clinical features	Age > 65 y		1				
	Constitutional symptoms				1	Constitutional symptoms	2
	Hemoglobin < 10 g/dL		2	Hemoglobin < 10 g/dL	1	Hemoglobin < 8 g/dL (women), < 9 g/dL (men)	2
						Hemoglobin 8–9.9 g/dL (women), 9–10.9 g/dL (men)	1
	Leukocytes > 25 G/L		1	Leukocytes > 25 G/L	2		
	Blasts in peripheral blood > 1%		1	Blasts in peripheral blood > 2%			1
		Transfusion dependency	1				
		Thrombocytes < 100 G/L	1	Thrombocytes < 100 G/L	2		
Karyotype		Unfavorable karyotype[a]	1			Unfavorable karyotype[c]	3
						VHR karyotype[c]	4
Fibrosis				Fibrosis grade 2 or 3	1		
Mutations				No *CALR* type 1 mutation	1	No *CALR* type 1 mutation	2
				HMR mutation[b]	1	HMR mutation[d]	2
				2 or more HMR mutations	2	2 or more HMR mutations	3
Interpretation (*median survival in years*)							
Very low						0 (*NR*)	
Low	0 (*NR*)	0 (*15.4*)		0–1 (*27.7*)		1–2 (*16.4*)	
Intermediate-1	1–2 (*14.2*)	1 (*6.5*)		Intermediate 2–4 (*7.1*)		Intermediate 3–4 (*7.7*)	
Intermediate-2	3–4 (*4.0*)	2–3 (*2.9*)					
High	5–6 (*1.5*)	4–6 (*1.3*)		5–12 (*2.3*)		5–8 (*4.1*)	
Very high						≥ 9 (*1.8*)	

Abbreviations: HMR, high molecular risk; LDH, lactate dehydrogenase; NR, not reached.
[a]Complex, +8, −7/7q-, i(17q), −5/5q-, 12p-, inv(3), or 11q23 rearrangement.
[b]*ASXL1, EZH2, SRSF2, IDH1/2*.
[c]"Very high risk (VHR)"—single/multiple abnormalities of −7, i(17q), inv(3)/3q21, 12p −/12p11.2, 11q −/11q23, or other autosomal trisomies not including +8/+9 (e.g., +21, +19); "favorable"—normal karyotype or sole abnormalities of 13q −, +9, 20q −, chromosome 1 translocation/duplication or sex chromosome abnormality including -Y; "unfavorable"—all other abnormalities.[60]
[d]*ASXL1, EZH2, SRSF2, IDH1/2*, and *U2AF1 Q157*.

currently in development.[4] Fedratinib, a *JAK2* inhibitor with additional activity toward *FLT3*, was approved by the U.S. Food and Drug Administration for intermediate- and high-risk MF in 2019 and approval in Europe and Switzerland is awaited. Fedratinib effectively reduces splenomegaly and symptom burden in both treatment-naive patients and patients resistant or intolerant to ruxolitinib.[73,74] The potential adverse event of Wernicke's encephalopathy led to a clinical hold of the JAKARTA phase 3 studies, but was subsequently attributed to poor nutrition status of the respective patients without clear association with fedratinib.[75] However, thiamin levels should be regularly checked in patients on fedratinib. The JAK1/2 inhibitor momelotinib evaluated by the SIMPLIFY phase 3 studies and currently under evaluation in the MOMENTUM trial showed beneficial effects not only for control of splenomegaly and constitutional symptoms, but also seems to improve anemia due to inhibition of hepatic hepcidin production.[76] The *JAK2*/*FLT3* inhibitor pacritinib currently in phase 3 studies seems to have advantageous profile for patients with thrombocytopenia.[77] Apart from novel *JAK2* inhibitors, several combination therapy approaches with ruxolitinib (e.g., with *Bcl2*/*Bcl-xL* inhibition) are in clinical development and novel therapeutic targets are also being addressed such as telomerase inhibition.[78–80] These novel approaches in development are reviewed in detail elsewhere.

Based on the rapid progress in the molecular understanding of MPNs in the last years, it is the goal and hope that new

innovative approaches for therapy will emerge from this insight and soon will become beneficial for MPN patients.

Conflict of Interest

F.C.Z. holds shares in Novartis. S.C.M. has consulted for and received honoraria from Celgene/BMS and Novartis.

References

1 Spivak JL. Myeloproliferative neoplasms. N Engl J Med 2017;376 (22):2168–2181
2 Rampal R, Al-Shahrour F, Abdel-Wahab O, et al. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. Blood 2014;123(22):e123–e133
3 Verstovsek S, Mesa RA, Gotlib J, et al; COMFORT-I Investigators. Long-term treatment with ruxolitinib for patients with myelofibrosis: 5-year update from the randomized, double-blind, placebo-controlled, phase 3 COMFORT-I trial. J Hematol Oncol 2017;10 (01):55
4 Brkic S, Meyer SC. Challenges and perspectives for therapeutic targeting of myeloproliferative neoplasms. HemaSphere 2020;5 (01):e516
5 Meyer SC, Levine RL. Molecular pathways: molecular basis for sensitivity and resistance to JAK kinase inhibitors. Clin Cancer Res 2014;20(08):2051–2059
6 Skoda RC, Duek A, Grisouard J. Pathogenesis of myeloproliferative neoplasms. Exp Hematol 2015;43(08):599–608
7 Jang MA, Seo MY, Choi KJ, Hong DS. A rare case of essential thrombocythemia with coexisting *JAK2* and *MPL* driver mutations. J Korean Med Sci 2020;35(23):e168
8 Beucher A, Dib M, Orvain C, et al. Next generation sequencing redefines a triple negative essential thrombocythaemia as double-positive with rare mutations on JAK2 V617 and MPL W515 hotspots. Br J Haematol 2019;186(05):785–788
9 James C, Ugo V, Le Couédic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005;434(7037):1144–1148
10 Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7(04):387–397
11 Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352(17):1779–1790
12 Baxter EJ, Scott LM, Campbell PJ, et al; Cancer Genome Project. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365(9464):1054–1061
13 Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 2007;356(05):459–468
14 Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med 2006;3(07):e270–e270
15 Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood 2006;108(10):3472–3476
16 Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med 2013;369(25):2379–2390
17 Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med 2013;369(25):2391–2405
18 Elf S, Abdelfattah NS, Chen E, et al. Mutant calreticulin requires both its mutant C-terminus and the thrombopoietin receptor for oncogenic transformation. Cancer Discov 2016;6(04): 368–381
19 Tefferi A, Lasho TL, Finke CM, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia 2014;28(07):1472–1477
20 Cabagnols X, Favale F, Pasquier F, et al. Presence of atypical thrombopoietin receptor (MPL) mutations in triple-negative essential thrombocythemia patients. Blood 2016;127(03):333–342
21 Lundberg P, Karow A, Nienhold R, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood 2014;123(14):2220–2228
22 Grinfeld J, Nangalia J, Baxter EJ, et al. Classification and personalized prognosis in myeloproliferative neoplasms. N Engl J Med 2018;379(15):1416–1430
23 Abdel-Wahab O, Pardanani A, Patel J, et al. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. Leukemia 2011;25(07):1200–1202
24 Rampal R, Ahn J, Abdel-Wahab O, et al. Genomic and functional analysis of leukemic transformation of myeloproliferative neoplasms. Proc Natl Acad Sci U S A 2014;111(50):E5401–E5410
25 Emanuel RM, Dueck AC, Geyer HL, et al. Myeloproliferative neoplasm (MPN) symptom assessment form total symptom score: prospective international assessment of an abbreviated symptom burden scoring system among patients with MPNs. J Clin Oncol 2012;30(33):4098–4103
26 Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391–2405
27 Thiele J, Kvasnicka HM, Müllauer L, Buxhofer-Ausch V, Gisslinger B, Gisslinger H. Essential thrombocythemia versus early primary myelofibrosis: a multicenter study to validate the WHO classification. Blood 2011;117(21):5710–5718
28 Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat Genet 2012;44(11):1179–1181
29 Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371(26):2488–2498
30 Malcovati L, Gallì A, Travaglino E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood 2017; 129(25):3371–3378
31 Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017; 377(02):111–121
32 McKerrell T, Park N, Chi J, et al. *JAK2* V617F hematopoietic clones are present several years prior to MPN diagnosis and follow different expansion kinetics. Blood Adv 2017;1(14):968–971
33 Barbui T, Tefferi A, Vannucchi AM, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. Leukemia 2018;32(05):1057–1069
34 Landolfi R, Marchioli R, Kutti J, et al; European Collaboration on Low-Dose Aspirin in Polycythemia Vera Investigators. Efficacy and safety of low-dose aspirin in polycythemia vera. N Engl J Med 2004;350(02):114–124
35 Marchioli R, Finazzi G, Specchia G, et al; CYTO-PV Collaborative Group. Cardiovascular events and intensity of treatment in polycythemia vera. N Engl J Med 2013;368(01):22–33
36 Barbui T, Masciulli A, Marfisi MR, et al. White blood cell counts and thrombosis in polycythemia vera: a subanalysis of the CYTO-PV study. Blood 2015;126(04):560–561
37 Gangat N, Strand J, Li CY, Wu W, Pardanani A, Tefferi A. Leucocytosis in polycythaemia vera predicts both inferior survival and leukaemic transformation. Br J Haematol 2007;138(03):354–358
38 Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. Leukemia 2010;24 (09):1574–1579

39 Barosi G, Birgegard G, Finazzi G, et al. A unified definition of clinical resistance and intolerance to hydroxycarbamide in polycythaemia vera and primary myelofibrosis: results of a European LeukemiaNet (ELN) consensus process. Br J Haematol 2010;148 (06):961–963

40 Kiladjian JJ, Cassinat B, Chevret S, et al. Pegylated interferon-alfa-2a induces complete hematologic and molecular responses with low toxicity in polycythemia vera. Blood 2008;112(08):3065–3072

41 Gisslinger H, Klade C, Georgiev P, et al; PROUD-PV Study Group. Ropeginterferon alfa-2b versus standard therapy for polycythaemia vera (PROUD-PV and CONTINUATION-PV): a randomised, non-inferiority, phase 3 trial and its extension study. Lancet Haematol 2020;7(03):e196–e208

42 Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med 2015;372(05):426–435

43 Passamonti F, Griesshammer M, Palandri F, et al. Ruxolitinib for the treatment of inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. Lancet Oncol 2017;18(01):88–99

44 Rocca B, Tosetto A, Betti S, et al. A randomized double-blind trial of 3 aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia. Blood 2020;136(02):171–182

45 Alvarez-Larrán A, Pereira A, Guglielmelli P, et al. Antiplatelet therapy versus observation in low-risk essential thrombocythemia with a CALR mutation. Haematologica 2016;101(08):926–931

46 Barbui T, Finazzi G, Carobbio A, et al. Development and validation of an international prognostic score of thrombosis in World Health Organization-essential thrombocythemia (IPSET-thrombosis). Blood 2012;120(26):5128–5133, quiz 5252

47 Haider M, Gangat N, Lasho T, et al. Validation of the revised international prognostic score of thrombosis for essential thrombocythemia (IPSET-thrombosis) in 585 Mayo Clinic patients. Am J Hematol 2016;91(04):390–394

48 Passamonti F, Thiele J, Girodon F, et al. A prognostic model to predict survival in 867 World Health Organization-defined essential thrombocythemia at diagnosis: a study by the International Working Group on Myelofibrosis Research and Treatment. Blood 2012;120(06):1197–1201

49 Carobbio A, Antonioli E, Guglielmelli P, et al. Leukocytosis and risk stratification assessment in essential thrombocythemia. J Clin Oncol 2008;26(16):2732–2736

50 Carobbio A, Ferrari A, Masciulli A, Ghirardi A, Barosi G, Barbui T. Leukocytosis and thrombosis in essential thrombocythemia and polycythemia vera: a systematic review and meta-analysis. Blood Adv 2019;3(11):1729–1737

51 Harrison CN, Campbell PJ, Buck G, et al; United Kingdom Medical Research Council Primary Thrombocythemia 1 Study. Hydroxyurea compared with anagrelide in high-risk essential thrombocythemia. N Engl J Med 2005;353(01):33–45

52 Gisslinger H, Gotic M, Holowiecki J, et al; ANAHYDRET Study Group. Anagrelide compared with hydroxyurea in WHO-classified essential thrombocythemia: the ANAHYDRET Study, a randomized controlled trial. Blood 2013;121(10):1720–1728

53 Gisslinger H, Buxhofer-Ausch V, Hodisch J, et al. A phase III randomized, multicentre, double blind, active controlled trial to compare the efficacy and safety of two different anagrelide formulations in patients with essential thrombocythaemia - the TEAM-ET 2·0 trial. Br J Haematol 2019;185(04):691–700

54 Harrison CN, Mead AJ, Panchal A, et al. Ruxolitinib vs best available therapy for ET intolerant or resistant to hydroxycarbamide. Blood 2017;130(17):1889–1897

55 O'Sullivan JM, Hamblin A, Yap C, et al. The poor outcome in high molecular risk, hydroxycarbamide-resistant/intolerant ET is not ameliorated by ruxolitinib. Blood 2019;134(23):2107–2111

56 Tefferi A. Primary myelofibrosis: 2021 update on diagnosis, risk-stratification and management. Am J Hematol 2021;96(01): 145–162

57 Passamonti F, Cervantes F, Vannucchi AM, et al. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). Blood 2010;115(09):1703–1708

58 Gangat N, Caramazza D, Vaidya R, et al. DIPSS plus: a refined dynamic international prognostic scoring system for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. J Clin Oncol 2011;29(04):392–397

59 Guglielmelli P, Lasho TL, Rotunno G, et al. MIPSS70: mutation-enhanced international prognostic score system for transplantation-age patients with primary myelofibrosis. J Clin Oncol 2018; 36(04):310–318

60 Tefferi A, Guglielmelli P, Lasho TL, et al. MIPSS70+ version 2.0: mutation and karyotype-enhanced international prognostic scoring system for primary myelofibrosis. J Clin Oncol 2018;36(17): 1769–1770

61 Tefferi A, Guglielmelli P, Nicolosi M, et al. GIPSS: genetically inspired prognostic scoring system for primary myelofibrosis. Leukemia 2018;32(07):1631–1642

62 Cervantes F, Isola IM, Alvarez-Larrán A, Hernández-Boluda JC, Correa JG, Pereira A. Danazol therapy for the anemia of myelofibrosis: assessment of efficacy with current criteria of response and long-term results. Ann Hematol 2015;94(11):1791–1796

63 Verstovsek S, Mesa RA, Gotlib J, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med 2012;366(09):799–807

64 Harrison C, Kiladjian J-J, Al-Ali HK, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med 2012;366(09):787–798

65 Verstovsek S, Gotlib J, Mesa RA, et al. Long-term survival in patients treated with ruxolitinib for myelofibrosis: COMFORT-I and -II pooled analyses. J Hematol Oncol 2017;10(01):156

66 Martínez-Trillos A, Gaya A, Maffioli M, et al. Efficacy and tolerability of hydroxyurea in the treatment of the hyperproliferative manifestations of myelofibrosis: results in 40 patients. Ann Hematol 2010;89(12):1233–1237

67 Geyer HL, Dueck AC, Scherber RM, Mesa RA. Impact of inflammation on myeloproliferative neoplasm symptom development. Mediators Inflamm 2015;2015:284706

68 Kleppe M, Kwak M, Koppikar P, et al. JAK-STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response. Cancer Discov 2015;5(03):316–331

69 Porpaczy E, Tripolt S, Hoelbl-Kovacic A, et al. Aggressive B-cell lymphomas in patients with myelofibrosis receiving JAK1/2 inhibitor therapy. Blood 2018;132(07):694–706

70 Tefferi A, Pardanani A. Serious adverse events during ruxolitinib treatment discontinuation in patients with myelofibrosis. Mayo Clin Proc 2011;86(12):1188–1191

71 Palandri F, Palumbo GA, Elli EM, et al. Ruxolitinib discontinuation syndrome: incidence, risk factors, and management in 251 patients with myelofibrosis. Blood Cancer J 2021;11(01):4

72 Kröger NM, Deeg JH, Olavarria E, et al. Indication and management of allogeneic stem cell transplantation in primary myelofibrosis: a consensus process by an EBMT/ELN international working group. Leukemia 2015;29(11):2126–2133

73 Pardanani A, Harrison C, Cortes JE, et al. Safety and efficacy of fedratinib in patients with primary or secondary myelofibrosis: a randomized clinical trial. JAMA Oncol 2015;1(05):643–651

74 Harrison CN, Schaap N, Vannucchi AM, et al. Fedratinib in patients with myelofibrosis previously treated with ruxolitinib: an updated analysis of the JAKARTA2 study using stringent criteria for ruxolitinib failure. Am J Hematol 2020;95(06):594–603

75 Mullally A, Hood J, Harrison C, Mesa R. Fedratinib in myelofibrosis. Blood Adv 2020;4(08):1792–1800

76 Harrison CN, Vannucchi AM, Platzbecker U, et al. Momelotinib versus best available therapy in patients with myelofibrosis

previously treated with ruxolitinib (SIMPLIFY 2): a randomised, open-label, phase 3 trial. Lancet Haematol 2018;5(02):e73–e81

77 Mascarenhas J, Hoffman R, Talpaz M, et al. Pacritinib vs best available therapy, including ruxolitinib, in patients with myelofibrosis: a randomized clinical trial. JAMA Oncol 2018;4(05): 652–659

78 Waibel M, Solomon VS, Knight DA, et al. Combined targeting of JAK2 and Bcl-2/Bcl-xL to cure mutant JAK2-driven malignancies and overcome acquired resistance to JAK2 inhibitors. Cell Rep 2013;5(04):1047–1059

79 Tefferi A, Lasho TL, Begna KH, et al. A pilot study of the telomerase inhibitor imetelstat for myelofibrosis. N Engl J Med 2015;373(10): 908–919

80 Baerlocher GM, Oppliger Leibundgut E, Ottmann OG, et al. Telomerase inhibitor imetelstat in patients with essential thrombocythemia. N Engl J Med 2015;373(10):920–928

