Role of tyrosine-kinase inhibitors in myeloproliferative neoplasms: comparative lessons learned

Javier Pinilla-Ibarz

Kendra L Sweet

Gabriela M Corrales-Yepe

Rami S Komrokji

Department of Malignant Hematology,
H Lee Moffitt Cancer Center
and Research Institute, Tampa,
FL, USA

Abstract: An important pathogenetic distinction in the classification of myeloproliferative neoplasms (MPNs) is the presence or absence of the *BCR-ABL* fusion gene, which encodes a unique oncogenic tyrosine kinase. The *BCR-ABL* fusion, caused by the formation of the Philadelphia chromosome (Ph) through translocation, constitutes the disease-initiating event in chronic myeloid leukemia. The development of successive *BCR-ABL*-targeted tyrosine-kinase inhibitors has led to greatly improved outcomes in patients with chronic myeloid leukemia, including high rates of complete hematologic, cytogenetic, and molecular responses. Such levels of treatment success have long been elusive for patients with Ph-negative MPNs, because of the difficulties in identifying specific driver proteins suitable as drug targets. However, in recent years an improved understanding of the complex pathobiology of classic Ph-negative MPNs, characterized by variable, overlapping multimutation profiles, has prompted the development of better and more broadly targeted (to pathway rather than protein) treatment options, particularly JAK inhibitors. In classic Ph-negative MPNs, overactivation of JAK-dependent signaling pathways is a central pathogenic mechanism, and mutually exclusive mutations in *JAK2*, *MPL*, and *CALR* linked to aberrant JAK activation are now recognized as key drivers of disease progression in myelofibrosis (MF). In clinical trials, the *JAK1/JAK2* inhibitor ruxolitinib – the first therapy approved for MF worldwide – improved disease-related splenomegaly and symptoms independent of *JAK2*^{V617F} mutational status, and prolonged survival compared with placebo or standard therapy in patients with advanced MF. In separate trials, ruxolitinib also provided comprehensive hematologic control in patients with another Ph-negative MPN – polycythemia vera. However, complete cytogenetic or molecular responses with JAK inhibitors alone are normally not observed, underscoring the need for novel combination therapies of JAK inhibitors and complementary agents that better address the complexity of the pathobiology of classic Ph-negative MPNs. Here, we discuss the role of tyrosine-kinase inhibitors in the current MPN-treatment landscape.

Keywords: chronic myeloid leukemia, myelofibrosis, tyrosine-kinase inhibitors, ruxolitinib, JAK inhibitor

Introduction

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of hematologic malignancies characterized by the increased clonal proliferation of one or more myeloid lineages.^{1,2} Classic MPNs include chronic myeloid leukemia (CML), primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocythemia (ET), whereas nonclassic MPNs include less common malignancies, such as chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia not otherwise specified

Correspondence: Javier Pinilla-Ibarz
Department of Malignant Hematology,
H Lee Moffitt Cancer Center and
Research Institute, 13131 Magnolia
Drive, Tampa, FL 33612, USA
Tel +1 813 745 4748
Fax +1 813 745 6817
Email javier.pinilla@moffitt.org

(CEL-NOS), mast-cell disease (mastocytosis), and MPNs unclassifiable.³ MPNs are further divided into two groups based on the presence or absence of the Philadelphia chromosome (Ph), formed by a translocation event and housing the constitutively activated fusion tyrosine kinase (TK) gene *BCR-ABL*.² *BCR-ABL* positivity is exclusively associated with CML, whereas Ph-negative MPNs are a heterogeneous group that includes all other MPNs.²

The identification of the *BCR-ABL* fusion gene in the pathogenesis of CML and the subsequent development of TK inhibitors (TKIs) that target the *BCR-ABL* fusion protein were major breakthroughs in cancer therapy and recognized as one of the best examples of rationally developed targeted drugs in oncology. Since then, characterization of mutations in other MPNs has improved the understanding of the pathogenesis of these diseases; however, unlike CML, the disease may be driven by more than one mutation or mechanism. Consequently, clinical development of specific TKIs has focused on targeting the abnormal signaling (eg, JAK pathway) involved in the pathogenesis of Ph-negative MPNs. The objective of this review is to provide the basis to understand the role of TKIs in the treatment of MPNs and why the mechanisms and benefits of therapeutic action differ between Ph-negative and Ph-positive diseases.

Pathobiology of MPNs

Classic Ph-positive MPNs: CML

CML is a myeloid malignancy originating from the clonal proliferation of a pluripotent stem cell in the bone marrow⁴ and involving one or all myeloid lineages.⁵ CML is an exemplary paradigm of a neoplasm that is essentially defined by a singular, unique cytogenetic abnormality, the Ph chromosome, resulting from a reciprocal chromosomal translocation between the long arms of chromosomes 9 and 22.⁶ The molecular consequence of this t(9;22) translocation is the creation of *BCR-ABL*, a chimeric protein with constitutive oncogenic TK activity.⁷ Expression of *BCR-ABL* results in the upregulation of multiple downstream oncogenic pathways,⁸ and has been shown to be the initiating event for the leukemogenesis of CML.⁹ Additionally, several lines of evidence indicate that disease progression from chronic phase (CP) to blast phase is driven by amplification of *BCR-ABL* in cooperation with other pathways, such as β -catenin.^{10,11}

CML has a triphasic natural history: the initial indolent CP precedes an accelerated phase and blast phase. Most patients are diagnosed in CP, with 20%–40% of newly diagnosed patients being asymptomatic at presentation.⁴ The

estimated yearly incidence of CML is approximately 1.8 per 100,000 persons in the US, accounting for about 15% of all adults with leukemia.^{12,13} Prior to the introduction of TKIs for CML, patients had a dismal prognosis, with an 8-year survival rate of 42%–65% from 1983–2000 for patients in CP.¹⁴

Classic Ph-negative MPNs: PV, ET, and PMF

PV, ET, and PMF are Ph-negative neoplastic hematopoietic stem cell disorders characterized by the uncontrolled clonal proliferation of myeloid progenitor cells affecting one or more myeloid lineages.^{1,2} These classic MPNs are relatively rare, and have a prevalence that varies from four to six per 100,000 persons for myelofibrosis (MF) to 45–57 and 39–57 per 100,000 persons for PV and ET, respectively.¹⁵ Although classic Ph-negative MPNs are distinct diseases, they have a common pathobiology of hyperactivation of the JAK–STAT pathway, and may share specific molecular and clinical features, including somatic stem cell mutations, elevated blood cell counts resulting from aberrant myeloproliferation, and constitutional symptoms. However, patients with ET, PV, and PMF differ regarding life expectancy at diagnosis, risk of leukemic transformation,¹⁶ rate of disease progression, and clinical presentation. Patients with any of these Ph-negative MPNs have a risk of progression to acute myeloid leukemia, which ranges from 2%–7% for PV and ET to 8%–30% for PMF.¹⁶ Patients with PV and ET have an approximate 10%–20% probability of evolving to post-PV MF or post-ET MF.¹⁶ In recognition of the largely indistinguishable clinical manifestations, clinical course, and histopathological features of PMF, post-PV MF, and post-ET MF,¹⁷ these diseases are collectively referred to as MF in this review.

The clinical presentation of MF is heterogeneous, with patients experiencing various degrees of bone marrow fibrosis, extramedullary hematopoiesis, spleen enlargement, anemia, and MF-related symptoms, many of which appear to be linked to the excessive production of inflammatory cytokines. The often-debilitating symptom burden of MF has a profound negative effect on quality of life and may shorten survival.^{18–20} PV is characterized by an increase in red blood cell production, low serum erythropoietin, and megakaryocyte pleomorphism in the bone marrow, whereas ET is associated with increased platelet production and increased megakaryocytic mass.^{21,22}

Unlike CML, classic Ph-negative MPNs are characterized by complex and variable mutation profiles and

Figure 1 Central role of JAK–STAT pathway in Ph-negative myeloproliferative neoplasms.

Note: Adapted from *Leuk Res*; 38(9); Savona MR; Are we altering the natural history of primary myelofibrosis?; 1001–1012; Copyright © 2014 The Author; Published by Elsevier Ltd; All rights reserved; with permission from Elsevier.¹⁷⁴

Abbreviation: TPO, thrombopoietin.

clonal evolution with unknown disease-initiating event(s) (Figure 1).²³ In the past decade, the identification and characterization of specific mutations associated with PV, ET, and PMF (Table 1) have begun to elucidate the underlying molecular pathobiology of classic Ph-negative MPNs and have prompted the development of targeted agents and disease-specific diagnostic tools.^{24–30}

Mutations affecting the JAK–STAT pathway

JAK2-mediated transduction of signals by activated hematopoietic cytokine receptors is crucial for hematopoiesis. The identification of the *JAK2*^{V617F} gain-of-function mutation in patients with classic Ph-negative MPNs³¹ provided the first clue to the pathogenic mechanism of these diseases. As a somatic mutation in hematopoietic stem cells that enables constitutive JAK2 activity in the absence of cytokines binding to their cognate receptors, this seemed to be an ideal candidate to explain the pathogenesis of MPNs, prompting numerous development efforts for JAK2-targeted therapies. However, patients without this mutation also develop Ph-negative MPNs and have evidence of increased JAK–STAT activity. The *JAK2*^{V617F} mutation is present in >95% of patients with PV and >50% of patients

with ET or PMF (Table 1).^{32–34} Moreover, even when present, the *JAK2*^{V617F} mutation does not appear to be sufficient as a disease-initiating event.²⁶

Intensive research in the last decade has not only revealed an unexpected genetic complexity of these diseases,³¹ but also suggests that aberrant JAK–STAT activation is a central defect, even in patients without the *JAK2*^{V617F} mutation.²⁸ Interestingly, disease-related hyperactivation of JAK–STAT signaling appears not to be limited to the mutant clonal stem cells, and is seen in “normal” nonclonal cells, particularly in MF, which is characterized by high circulating levels of inflammatory cytokines. The postulated role of JAK–STAT signaling in maintaining a state of excess inflammation and catabolism that promotes constitutional symptoms and cachexia appears to be validated by clinical evidence showing that JAK inhibition substantially reduces the levels of inflammatory cytokines, reverses signs of cachexia (eg, weight loss), and significantly reduces symptom burden compared with placebo or conventional therapy.^{35,36}

In addition to *JAK2*^{V617F}, a number of mutations have been identified that lead to hyperactivation of the JAK–STAT pathway in classic Ph-negative MPNs (Table 1).³⁷ Approximately 3% of all patients with PV have mutations

Table 1 Common mutations in classic Ph-negative MPNs

Mutation	Frequency of mutation	Prognostic relevance
Mutations affecting JAK–STAT signaling		
<i>JAK2</i> ^{V617F} ^{32,34,158,159}	PV: ~96% ET: ~55% PMF: ~65%	<ul style="list-style-type: none"> Potentially higher risk of thrombotic and vascular complications in ET Increased risk of fibrotic transformation in ET and PV Leukocytosis and larger splenomegaly in PV, ET, and PMF Course of PV disease similar to <i>JAK2</i>^{V617F} PV
<i>JAK2</i> exon 12 ^{34,38,160}	PV: ~3% ET: rare PMF: rare	
<i>MPL</i> exon 10 ^{34,39,161,162}	PV: rare ET: ~3% PMF: ~11%	<ul style="list-style-type: none"> Higher risk of postdiagnosis arterial thrombosis in ET Lower hemoglobin level and higher risk of transfusion dependence in MF
<i>CALR</i> exon 9 ^{26,40,41,46}	PV: none ET: 15%–32% PMF: 25%–35%	<ul style="list-style-type: none"> Higher platelet count in patients with ET More indolent disease phenotype in PMF, with lower risk of thrombosis and blast transformation and longer overall survival
Somatic mutations in epigenetic modifiers		
<i>TET2</i> , any of 12 exons ^{26,34,163}	PV: ~16% ET: ~5% PMF: ~17% Post-PV MF: ~14% Post-ET MF: ~14%	<ul style="list-style-type: none"> Increased rates of leukemic transformation
<i>ASXL1</i> exon 12 ^{24,25,34,164}	PV: ~7% ET: ~4% MF: ~20%	<ul style="list-style-type: none"> Poor survival and increased rates of leukemic transformation
<i>IDH1/IDH2</i> exon 4 ^{25,65,165}	PV: ~2% ET: ~1% PMF: ~4% Post-PV MF: ~1% Post-ET MF: ~1%	<ul style="list-style-type: none"> Increased rates of leukemic transformation and shorter survival Higher rates of leukemic transformation for patients with concomitant <i>JAK2</i>^{V617F} mutation
<i>EZH2</i> mutations occur across the gene's 20 exons; intronic mutations have been reported ^{166,167}	PMF: ~6% Post-PV MF: ~1% Post-ET MF: ~9%	<ul style="list-style-type: none"> Increased rates of leukemic transformation and shorter survival
<i>DNMT3A</i> methyltransferase domain ^{45,168,169}	PV: ~7% ET: ~3% MF: ~15%	<ul style="list-style-type: none"> Unclear prognostic value in MPNs

Abbreviations: ET, essential thrombocytopenia; MF, myelofibrosis; MPN, myeloproliferative neoplasm; PMF, primary myelofibrosis; PV, polycythemia vera.

in *JAK2* exon 12,^{34,38} and patients with PMF or ET may have mutations in the thrombopoietin-receptor gene *MPL*³⁹ or in the *CALR* gene. With few exceptions, *CALR*, *JAK2*, and *MPL* mutations appear to be mutually exclusive within the same patient.^{24,26,40,41} Although the precise mechanism of action of the *CALR* mutation remains unclear, evidence from an integrated genomic analysis²⁸ and preclinical studies^{26,41} suggests that *CALR* mutations facilitate cytokine-independent JAK–STAT pathway activation. This provides a strong rationale for the use of JAK-inhibitor therapy in MPN patients with *CALR* mutations, and may at least in part be responsible for the clinical responses to JAK-inhibitor therapy observed in patients with *JAK2*^{V617F}-negative MF.^{29,35,36,42,43}

Mutations in epigenetic modifiers

Genetic aberrations present in patients with classic Ph-negative MPNs often include somatic mutations in epigenetic

modifiers, such as *TET2*, *ASXL1*, *DNMT3A*, *EZH2*, or *IDH1* (Table 1), which may be acquired prior to or after *JAK2*^{V617F} or *CALR* mutations. Recent evidence suggests that the order of acquiring mutations may determine the specific MPN phenotype that a patient develops.⁴⁴ Although no particular epigenetic mutation has a reported frequency >20%, overall, mutations in genes encoding epigenetic regulators appear to be common in classic Ph-negative MPNs. The prognostic and clinical relevance of these mutations in different MPNs is still being elucidated,^{26,32} however, there is evidence that some mutations in epigenetic modifiers may increase the risk of leukemic transformation and/or shorten survival.^{26,45} Moreover, the number of mutations occurring in addition to those in *JAK2*, *MPL*, or *CALR* has been shown to affect outcomes. Patients harboring more than one mutation have a markedly increased probability of leukemic transformation and shortened survival.²⁶ In patients with MF,

“triple-negative” mutation status (ie, no mutation in *JAK2*, *MPL*, or *CALR*) is associated with the worst outcome, and the presence of *ASXL1* mutation is an additional independent prognostic factor for poor survival.^{24,46}

Nonclassic Ph-negative MPNs

Nonclassic MPNs are less common Ph-negative hematologic disorders that include CNL, CEL-NOS, mastocytosis, and MPNs unclassifiable.^{3,47} Recent discoveries of genetic abnormalities in nonclassic Ph-negative MPNs have paved the way for the development of targeted therapies that may impact the therapeutic approach to these rarer diseases that have historically had few treatment options.

Mutations in the *CSF3R* gene have recently been identified in 59% of patients with CNL or atypical CML (aCML).⁴⁸ Functional genomic data indicate the existence of two types of *CSF3R* mutations with differential downstream effects: truncation mutations that activate the TNK2 and Src pathways, and membrane-proximal mutations that activate the JAK–STAT pathway.⁴⁸ This has provided the rationale for the investigation of JAK or Src inhibitors to target membrane-proximal or truncation *CSF3R* mutations, respectively, in patients with CNL and aCML.⁴⁸

Approximately 93% of adult patients with indolent and aggressive systemic mastocytosis carry the *KIT*^{D816V} mutation, which results in constitutive activation of the KIT TK and unrestricted cellular proliferation.^{49,50} Presence of this mutation is associated with higher bone marrow mast-cell burden and the presence of C-findings, including marrow dysfunction with cytopenias, hepatomegaly, splenomegaly, osteolysis, pathological fractures, weight loss, and leukemic transformation, which may be indicative of poor prognosis.⁵¹ Additional pathways also have been implicated in the pathobiology of the disease, supporting the rationale for a multitargeted therapeutic approach.⁵⁰

Compared with the pathogenesis of CML, which is essentially driven by a de novo oncoprotein resulting from a unique chromosomal translocation, the pathobiology of classic Ph-negative MPNs is both variable and highly complex, often involving point mutations in multiple genes that are difficult to target without affecting nononcogenic pathways. The *JAK2*^{V617F} mutation found in the majority of patients with these diseases is neither essential nor specific for any of these MPNs. Moreover, the *JAK2*^{V617F} mutation is not a disease-initiating event, and many questions remain regarding the precise pathogenic mechanisms of other mutations. However, dysregulation of JAK–STAT signaling in and beyond hematopoietic stem cells is a dominant defect

in classic Ph-negative MPNs, and current evidence validates the continued targeting of JAK–STAT signaling in MPNs, even in patients without mutations in *JAK2*.

Because of the overall lack of specificity of patients' mutation profiles and overlapping clinical features at presentation, particularly at early disease stages, a combination of genetic, clinical, cytogenetic, and histomorphologic criteria is required for the definitive diagnosis of classic MPNs.

Diagnostic criteria

CML

Detectable presence of the Ph chromosome is required to confirm a CML diagnosis and to distinguish it from all other MPNs.⁵² The initial evaluation of patients with suspected CML should include physical examination, including spleen measurements, complete blood count with differential, chemistry profile, bone marrow morphology and cytogenetics, and real-time quantitative polymerase chain reaction (PCR) using the international scale for *BCR–ABL1* (Table 2).^{4,12} Most patients are diagnosed in CP and have distinct laboratory features, such as leukocytosis with all stages of maturation, basophilia, thrombocytosis, and hypercellularity in the bone marrow.⁴

Although the presence of *BCR–ABL* can be assessed by fluorescence in situ hybridization or quantitative reverse-transcriptase PCR in the peripheral blood, cytogenetic evaluation of bone marrow aspirate is recommended to detect cytogenetic abnormalities and perform accurate staging into the chronic phase, accelerated phase, or blast phase.^{12,53} Multiple staging criteria for CML have been developed; however, the different thresholds used to distinguish between the chronic phase, accelerated phase, and blast phase have not been validated in clinical trials. Most CML trials have used the staging system developed by the MD Anderson Cancer Center, which defines accelerated phase as $\geq 15\%$ blasts, $\geq 30\%$ blasts plus promyelocytes, $\geq 20\%$ basophils in blood or bone marrow, platelets $< 100 \times 10^9/L$ unrelated to therapy, or cytogenetic clonal evolution; and the blast phase as $\geq 30\%$ blasts in blood or bone marrow in the presence of extramedullary disease with localized immature blasts.^{53,54}

Ph-negative MPNs

Diagnosis of classic Ph-negative MPNs is based on the evaluation of clinical, histopathologic, morphologic, molecular, and cytogenetic variables (Table 2).³ Unlike the presence of *BCR–ABL* (which is sufficient for a CML diagnosis), the presence of *JAK2* mutations is used to support the diagnosis of Ph-negative MPNs, but cannot differentiate between subtypes.³

Table 2 Diagnostic criteria for classic MPNs

Disease	Major criteria	Minor criteria
Ph-positive classic MPNs¹⁷⁰		
Chronic myeloid leukemia	<ul style="list-style-type: none"> Positive for BCR-ABL1 	<ul style="list-style-type: none"> Constitutional symptoms: <ul style="list-style-type: none"> Fatigue Weight loss Night sweats Splenomegaly Anemia Leukocytosis (median WBC count $\sim 100 \times 10^9/L$; range $12-1,000 \times 10^9/L$) Neutrophils in different stages of maturation, with predominant segmented neutrophils and myelocytes Presence of absolute basophilia Normal or elevated platelet count (rare thrombocytopenia) BM granulocytic proliferation, leading to increased cellularity Blasts $< 5\%$ of nucleated BM cells BM erythroid islands reduced in number and size Smaller and megakaryocytes with hypolobated nuclei (dwarf megakaryocytes) in BM Moderate or marked reticulin fibrosis in BM
Ph-negative classic MPNs¹⁷¹		
PMF ^a	<ul style="list-style-type: none"> Megakaryocyte proliferation and atypia^a accompanied by either reticulin and/or collagen fibrosis^e Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm Demonstration of JAK2^{V617F} or other clonal marker or no evidence of reactive marrow fibrosis Hb > 18.5 g/dL (men), > 16.5 g/dL^f Presence of JAK2^{V617F} or JAK2 exon 12 mutation 	<ul style="list-style-type: none"> Leukoerythroblastosis Increased serum LDH level Anemia Palpable splenomegaly
PV ^b	<ul style="list-style-type: none"> Platelet count $\geq 450 \times 10^9/L$ Megakaryocyte proliferation with large and mature morphology Not meeting WHO criteria for CML, PV, PMF, MDS, or other myeloid neoplasm Demonstration of JAK2^{V617F}/other clonal marker or no evidence of reactive thrombocytosis 	<ul style="list-style-type: none"> BM trilineage myeloproliferation Subnormal serum erythropoietin level EEC growth
Essential thrombocythemia ^c		
Ph-negative nonclassic MPNs⁴⁷		
Systemic mastocytosis ^d	<ul style="list-style-type: none"> Multifocal, dense infiltrates of mast cells (≥ 15 mast cells in aggregates) detected in sections of BM and/or other extracutaneous organ(s) 	<ul style="list-style-type: none"> In biopsy sections of BM or other extracutaneous organs, $> 25\%$ of the mast cells in the infiltrate are spindle-shaped or have atypical morphology, or of all mast cells in BM aspirate smears, $> 25\%$ are immature or atypical Detection of an activating mutation point at codon 816 of KIT in BM, blood, or another extracutaneous organ Mast cells in BM, blood, or other extracutaneous organs express CD2 and/or CD25, in addition to normal mast-cell markers Serum total tryptase persistently > 20 ng/mL (parameter valid only in the absence of an associated clonal myeloid disorder)

CNL

- Peripheral blood leukocytosis (WBC count $\geq 25 \times 10^9/L$) composed of: $>80\%$ segmented neutrophils and band forms; $<10\%$ immature granulocytes (promyelocytes, myelocytes, metamyelocytes); $<1\%$ myeloblasts
- Hypercellular BM biopsy consisting of: neutrophilic granulocytes increased in number and percentage; myeloblasts $<5\%$ of nucleated BM cells; neutrophilic maturation pattern normal; megakaryocytes normal or left-shifted
- Hepatosplenomegaly
- No identifiable cause for physiologic neutrophilia, or if present demonstration of clonality of myeloid cells by cytogenetic or molecular studies; no infectious or inflammatory process; no underlying tumor
- No Ph chromosome or *BCR-ABL1* fusion gene
- No rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*
- No evidence of PV, ET, PMF, MDS, or MDS/MPN
- No granulocytic dysplasia or myelodysplastic changes in other myeloid lineages

CEL-NOS

- Monocytes $<1 \times 10^9/L$
- Eosinophils $\geq 1.5 \times 10^9/L$
- Not meeting criteria for CML, PV, ET, MF, or MDS/MPN (CMML or aCML)
- No *t(5;12)(q31-q35;p13)* or any other rearrangement of *PDGFRB*
- No *FIP/IL1-PDGFRB* fusion gene or any other rearrangement of *PDGFRA*
- No rearrangement of *FGFR1*
- Blast count in the peripheral blood and BM $<20\%$
- No *inv(16)(p13.1;q22)* or *t(16;16)(p13.1;q22)* or other feature diagnostic of AML
- Clonal cytogenetic or molecular genetic abnormality present, or blast cells are $>2\%$ in the peripheral blood or $>5\%$ in the BM

Notes: ^aDiagnosis of PMF requires meeting all three major criteria and two minor criteria; ^bdiagnosis of PV requires meeting either both major criteria and one minor criterion or the first major criterion and two minor criteria; ^cdiagnosis of ET requires meeting all four major criteria; ^dsmall-to-large megakaryocytes with aberrant nuclear:cytoplasmic ratio, hyperchromatic and irregularly folded nuclei, and dense clustering; ^ein the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (ie, prefibrotic PMF); ^fHb or hematocrit >99 th percentile of reference range for age, sex, or altitude of residence or red cell mass $>25\%$ above mean normal (predicted or $Hb > 17$ g/dL (men) > 15 g/dL (women)) if associated with a sustained increase of ≥ 2 g/dL from baseline that cannot be attributed to correction of iron deficiency; ^gdiagnosis of systemic mastocytosis requires either the major criterion and one minor criterion or at least three minor criteria.

Abbreviations: aCML, atypical chronic myeloid leukemia; AML, acute myeloid leukemia; BM, bone marrow; CEL-NOS, chronic eosinophilic leukemia not otherwise specified; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; CNL, chronic neutrophilic leukemia; EEC, endogenous erythroid colony; ET, essential thrombocythemia; Hb, hemoglobin; LDH, lactate dehydrogenase; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; Ph, Philadelphia; PMF, primary myelofibrosis; PV, polycythemia vera; WBC, white blood cell; WHO, World Health Organization.

Owing to the lack of strictly disease-specific mutations in these MPNs and the possibility of phenotypic resemblance at early disease stage, histopathologic and morphologic examination of the bone marrow is necessary for a definitive diagnosis of ET and PMF.³ In addition, although bone marrow histomorphology is a minor diagnostic criterion for PV, a bone marrow biopsy showing age-adjusted hypercellularity with trilineage growth (panmyelosis) is very specific for PV and necessary to exclude reactive polycythemia in patients with *JAK2* mutation-negative PV³ and ET in patients with masked PV.⁵⁵

Nonclassic Ph-negative MPNs

Diagnosis of nonclassic MPNs can be difficult, and generally requires absence of BCR–ABL, dyserythropoiesis, and granulocyte dysplasia.^{3,47} Additional molecular and histologic parameters are used to discern between nonclassic MPNs, with particular focus on morphology, which is a key criterion in the diagnosis of these rare malignancies. The major criteria for diagnosis are listed in Table 2. Notably, many major criteria mandate confirmation that the patient has no other Ph-negative MPN or CML.

Prognostic risk stratification CML

Certain clinical characteristics at diagnosis may have prognostic value in patients with CML, and may guide optimal treatment selection and clinical management of the disease.⁵⁶ For over 30 years, prognostic scoring systems for risk stratification of patients with CML have been widely used to predict outcome. Their development reflects the increased understanding of the disease and the evolution of therapeutic strategies available for patients through the years, from conventional therapy in the 1980s to the use of TKIs in the new millennium.⁵⁶ Current prognostic scoring systems, including the Sokal, Hasford (also known as Euro), and EUTOS systems, classify patients into low-, intermediate-, and high-risk groups, although the EUTOS system does not include a definition for the intermediate-risk category (Table 3).^{57–59}

The Sokal and Hasford scoring systems were developed before the introduction of TKIs, and account for a patient's age, spleen size, platelet count, and percentage of blasts at diagnosis.^{57,59} In addition to these categories, the Hasford system also includes percentage of basophils and eosinophils.^{57,59} In a study of 813 patients diagnosed between 1962 and 1981, the Sokal system estimated a median survival of 60 and 30 months for low- and high-risk patients, respectively.⁵⁷

Similar results were obtained with the Hasford system in a meta-analysis of 1,303 patients treated between 1983 and 1994, which estimated median survival of 96 and 42 months for low- and high-risk patients, respectively.⁵⁹ The EUTOS scoring system is the latest prognostic tool, and was developed after imatinib therapy became the standard of care. This system considers only spleen size and basophil count at the time of diagnosis.⁵⁸

The prognostic validity of the Sokal, Hasford, and EUTOS systems needs further clarification, given the current use of TKIs as the standard of care in CML. In a study comparing the three scoring systems in 2,060 patients receiving first-line imatinib, the EUTOS scoring system proved to be superior to the Sokal and Hasford systems in predicting 5-year progression-free survival (PFS) and the probability of reaching complete cytogenetic response (CCyR) at 18 months.⁵⁸ Similarly, in two additional studies of front-line imatinib in 1,288 and 143 patients with CML, the EUTOS score was superior to the Sokal and Hasford scoring systems in predicting CCyR, event-free survival, and overall survival.^{60,61} However, another study in patients with early CP CML receiving first-line imatinib or second-generation TKIs did not find a correlation between the EUTOS risk stratification and outcomes.⁶² Currently, only the Sokal and Hasford scoring systems are recommended by the National Comprehensive Cancer Network (NCCN) guidelines.¹² These scoring systems may require additional updates, given that the prognostic parameters in the current scoring systems are limited and do not account for additional genetic or molecular biomarkers.⁵⁶

Ph-negative MPNs

Risk stratification of patients with PMF is determined according to the International Prognostic Scoring System (IPSS) at the time of diagnosis, and according to the dynamic IPSS (DIPSS) at any time during the course of the disease (Table 4).^{20,63} Both the IPSS and DIPSS estimate survival of patients with PMF by accounting for five independent factors: age >65 years, hemoglobin <10 g/dL, leukocyte count >25×10⁹/L, peripheral blood blasts ≥1%, and presence of disease-related constitutional symptoms. In addition, the DIPSS assigns higher prognostic weight to the acquisition of anemia over time, given its negative effect on survival compared with all other parameters.^{20,63} A third prognostic tool is the DIPSS Plus scoring system, which predicts overall survival in patients with PMF by combining the risk factors used in the DIPSS with platelet count <100×10⁹/L, transfusion dependence, and unfavorable karyotype during disease course. Additionally, unfavorable karyotype and platelet

Table 3 Risk stratification systems for chronic myeloid leukemia

	Sokal ⁵⁷	Hasford (Euro) ⁵⁸	EUTOS ⁵⁹
Year introduced	1984	1998	2011
Predominant treatment modality	Conventional chemotherapy	IFN α -based regimens	Imatinib
Factors	<ul style="list-style-type: none"> • Age • Spleen size • Platelet count • Percentage of blasts 	<ul style="list-style-type: none"> • Age • Spleen size • Platelet count • Percentage of blasts • Percentage of basophils • Percentage of eosinophils 	<ul style="list-style-type: none"> • Spleen size • Basophil count
Risk calculation	<ul style="list-style-type: none"> • The score is calculated using the following formula: $\exp(0.0116 \times (\text{age} [\text{years}] - 43.4)) + (0.0345 \times (\text{spleen size} [\text{cm}] - 7.51) + (0.188 \times (\text{platelets} [10^9/\text{L}] / 700)^2 - 0.563)) + (0.0887 \times (\text{blasts} [\%] - 2.10))$ 	<ul style="list-style-type: none"> • The score is calculated using the following formula: $(0.6666 \times \text{age} [0 \text{ when age} < 50 \text{ years; } 1 \text{ otherwise}]) + (0.0420 \times \text{spleen size} [\text{cm}]) + (0.0584 \times \text{blasts} [\%]) + (0.0413 \times \text{eosinophils} [\%]) + (0.2039 \times \text{basophils} [0 \text{ when basophils} < 3\%; 1 \text{ otherwise}]) + (1.0956 \times \text{platelet count} [0 \text{ when platelets} < 1500 \times 10^9/\text{L}; 1 \text{ otherwise}]) \times 1000$ 	<ul style="list-style-type: none"> • The score is calculated using the following formula: $(7 \times \text{basophil} [\%]) + (4 \times \text{spleen} [\text{cm}])$
Risk groups ^a	<ul style="list-style-type: none"> • High: score > 1.2 • Intermediate: score $0.8 - 1.2$ • Low: score < 0.8 	<ul style="list-style-type: none"> • High: score $> 1,480$ • Intermediate: score > 780 and $\leq 1,480$ • Low: score ≤ 780 	<ul style="list-style-type: none"> • High: score > 87 • Low: score ≤ 87

Note: ^aAn intermediate-risk category was not defined for EUTOS.

count $< 100 \times 10^9/\text{L}$ have been demonstrated to be independent predictors of leukemia-free survival and leukemic transformation.⁶⁴ All three prognostic scoring systems divide patients into four distinct risk categories according to each individual score: low-, intermediate 1-, intermediate 2-, or high-risk.^{20,63} However, the dynamic risk assessment provided by the DIPSS or DIPSS Plus may be a more valuable tool than the IPSS in guiding clinical choices that accurately address the patient's disease course.⁶³ Despite disease similarities between de novo and transformed MF and the common use

of prognostic models for PMF to drive treatment decisions in patients with post-ET MF or post-PV MF, recent research has suggested that the IPSS may not accurately discriminate different risk categories in patients with post-ET MF or post-PV MF.^{24,27,65} A separate scoring system may be warranted to aid physicians in treatment of these patients.⁶⁶ In addition, recent studies suggest that the specific mutation profiles may have a significant effect on prognosis in patients with PMF.^{24,27,65}

Owing to the increased risk of potentially fatal thrombosis and of microvascular vasomotor disturbances associated with

Table 4 Risk stratification for patients with classic Ph-negative myeloproliferative neoplasms

	ET ⁶⁹	PV ⁷⁰	PMF IPSS ²⁰	DIPSS ⁶³	DIPSS Plus ⁶⁴
Factors	<ul style="list-style-type: none"> • Age ≥ 60 years: 2 points • Leukocyte count $\geq 11 \times 10^9/\text{L}$: 1 point • History of thrombosis: 1 point 	<ul style="list-style-type: none"> • Age > 70 years • Leukocyte count $> 13 \times 10^9/\text{L}$ • History of thrombosis 	<ul style="list-style-type: none"> • Age > 65 years: 1 point • Hb $< 10 \text{ g/dL}$: 1 point • WBC count $> 25 \times 10^9/\text{L}$: 1 point • Blood blasts $> 1\%$: 1 point • Constitutional symptoms: 1 point 	<ul style="list-style-type: none"> • Age > 65 years: 1 point • Hb $< 10 \text{ g/dL}$: 2 points • WBC count $> 25 \times 10^9/\text{L}$: 1 point • Blood blasts $\geq 1\%$: 1 point • Constitutional symptoms: 1 point 	<ul style="list-style-type: none"> • DIPSS intermediate 1- risk: 1 point • Unfavorable karyotype: 1 point • Platelets $< 100 \times 10^9/\text{L}$: 1 point • Red cell transfusion need: 1 point • DIPSS intermediate 2- risk: 2 points • DIPSS high-risk: 3 points
Risk group	<ul style="list-style-type: none"> • Low: score 0 • Intermediate: score 1–2 • High: score > 2 	<ul style="list-style-type: none"> • Low: 0 factors • Intermediate: 1 factor • High: 2–3 factors 	<ul style="list-style-type: none"> • Low: score 0 • Intermediate 1: score 1 • Intermediate 2: score 2 • High: score > 2 	<ul style="list-style-type: none"> • Low: score 0 • Intermediate 1: score 1–2 • Intermediate 2: score 3–4 • High: score > 4 	<ul style="list-style-type: none"> • Low: score 0 • Intermediate 1: score 1 • Intermediate 2: score 2–3 • High: score > 3

Note: Risk group determined using the sum of point values from the individual factors.

Abbreviations: DIPSS, Dynamic International Prognostic Scoring System; ET, essential thrombocythemia; Hb, hemoglobin; IPSS, International Prognostic Scoring System; Ph, Philadelphia chromosome; PMF, primary myelofibrosis; PV, polycythemia vera; WBC, white blood cell.

ET and PV, prognostic stratification for these diseases aims to estimate the probability of thrombotic complications.^{67,68} The International Prognostic Score for ET predicts survival based on age, leukocyte count, and history of thrombosis (Table 4). According to the IPSS for ET, patients with ET are divided into three risk categories associated with substantial differences in survival: low-risk (median survival not reached), intermediate-risk (median survival 24.5 years), and high-risk (median survival 13.8 years).⁶⁹ Patients with PV are categorized as high- or low-risk based on age, leukocyte count, and history of previous thrombosis.⁷⁰ Abnormal karyotype has recently emerged as an additional prognostic factor correlated with shortened survival in these patients, but is not currently considered in prognostic models.⁷¹

TKI-based therapies for MPNs CML

Owing to the involvement of BCR–ABL in disease initiation and progression, targeted therapeutic strategies have focused on the development of small molecules that inhibit the oncogenic constitutive activation of this TK. The role of BCR–ABL as the principal driver of CML has led to a high treatment success rate with these targeted inhibitors.

Imatinib

Imatinib was the first BCR–ABL inhibitor approved in 2001 for the treatment of patients with blast crisis, accelerated phase, or CP CML who failed IFN α therapy, and was

subsequently approved in 2002 for newly diagnosed CP CML (Table 5).⁷² Imatinib was compared with standard IFN α plus low-dose cytarabine in the Phase III IRIS study of 1,106 patients with newly diagnosed CP CML.⁷³ At the 18-month follow-up, imatinib was superior to IFN α plus low-dose cytarabine in terms of response (complete hematologic response [CHR], major CyR [MCyR], and CCyR), decreased likelihood of progression to accelerated phase or blast crisis, and tolerability (Table 6). Durable response rates, decreased progression to more advanced phases, and an overall survival benefit were maintained with long-term imatinib therapy compared with standard chemotherapy.^{74,75}

Despite these promising results, an estimated 20%–30% of patients who achieve CHR and/or CCyR eventually develop secondary (acquired) resistance to imatinib.^{76,77} Approximately 40%–90% of imatinib-resistant patients develop secondary resistance as a result of mutations arising in the BCR–ABL kinase domain. These genetic aberrations include the T315I mutation, which changes the conformation of BCR–ABL, thus preventing imatinib from accessing the protein's adenosine triphosphate-binding pocket.^{76–80} Furthermore, several additional BCR–ABL-independent mechanisms of imatinib resistance, such as intracellular drug uptake, Src overexpression, leukemic stem cell quiescence, and clonal evolution, are currently under investigation.^{76,77} Second- and third-generation TKIs, such as dasatinib, nilotinib, bosutinib, and ponatinib were designed to overcome imatinib resistance.

Table 5 Approved therapies and their indication for patients with MPNs in the US

Drug	Mechanism of action	US indications for MPNs
Imatinib ⁷²	BCR–ABL inhibitor	<ul style="list-style-type: none"> Newly diagnosed adult and pediatric patients with Ph⁺ CML in chronic phase Patients with Ph⁺ CML in blast crisis, accelerated phase, or in chronic phase after failure of IFNα therapy
Dasatinib ⁸⁵	BCR–ABL inhibitor, including imatinib-resistant forms	<ul style="list-style-type: none"> Newly diagnosed adults with Ph⁺ CML in chronic phase Adults with chronic, accelerated, or myeloid or lymphoid blast phase Ph⁺ CML with resistance or intolerance to prior therapy, including imatinib
Nilotinib ⁸⁷	BCR–ABL inhibitor, preferentially mutant forms	<ul style="list-style-type: none"> Newly diagnosed adult patients with Ph⁺ CML in chronic phase Chronic phase and accelerated phase Ph⁺ CML in adult patients resistant to or intolerant to prior therapy that included imatinib
Bosutinib ⁹⁵	Dual BCR–ABL/Src inhibitor, including mutant BCR–ABL resistant to other TKIs	<ul style="list-style-type: none"> Adult patients with chronic phase, accelerated phase, or blast phase Ph⁺ CML with resistance or intolerance to prior therapy
Ponatinib ¹⁰³	Inhibitor of BCR–ABL, including T315I-mutated BCR–ABL	<ul style="list-style-type: none"> Adult patients with T315I-positive CML (chronic phase, accelerated phase, or blast phase) Adult patients with chronic phase, accelerated phase, or blast phase CML for whom no other TKI therapy is indicated
Ruxolitinib ¹¹⁰	JAK1/JAK2 inhibitor	<ul style="list-style-type: none"> Patients with intermediate- or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis Patients with polycythemia vera who have had an inadequate response to or are intolerant of hydroxyurea

Abbreviations: CML, chronic myeloid leukemia; MPNs, myeloproliferative neoplasms; Ph⁺, Philadelphia chromosome–positive; TKIs, tyrosine-kinase inhibitors.

Table 6 Primary results of Phase III trials of tyrosine kinase inhibitors for patients with newly diagnosed chronic phase–chronic myeloid leukemia

TKI	Study	CCyR (0 Ph ⁺ cells measured by either conventional or FISH cytogenetic testing)	MMR (≥ 3 -log reduction of BCR–ABL mRNA)	Progression to blast crisis	Kaplan–Meier estimated overall survival	Safety
Imatinib ⁷³	IRIS (N=1,106) Imatinib vs standard IFN α plus low-dose cytarabine	76.2% vs 14.5% ($P<0.001$) ^a at 18 months		Freedom from progression: 96.7% vs 91.5% ($P<0.001$) at 18 months	97.2% vs 95.1% ($P=0.16$) at 18 months	Imatinib was better tolerated than combination therapy; grade 3/4 AEs that were more common in the combination group included fatigue, depression, myalgia, arthralgia, neutropenia, and thrombocytopenia
Dasatinib ⁸⁴	DASISION (N=519) Dasatinib 100 mg QD vs imatinib 400 mg QD	77% vs 66% confirmed CCyR ($P=0.007$) by 12 months	46% vs 28% ($P<0.0001$) by 12 months	1.9% vs 3.5% at 12 months	97% vs 99% at 12 months	Similar rates, mostly grade 1 or 2; nausea, vomiting, fluid retention, and superficial edema more common with imatinib; pleural effusion only reported with dasatinib
Nilotinib ⁹²	ENESTnd (N=846) Nilotinib 300 mg BID and 400 mg BID vs imatinib 400 mg QD	80% and 78% vs 65% ($P<0.001$)	44% and 43% vs 22% ($P<0.001$)	<1% and <1% vs 4% ($P\leq 0.01$) at 12 months	Not reported at 12 months; 94.3% and 96.7% vs 93.3% at 48 months ⁹³	Dermatologic events and headache more common with nilotinib; GI events and fluid retention more common with imatinib
Bosutinib ^{b,102}	BELA (N=502) Bosutinib 500 mg/day vs imatinib 400 mg QD	70% vs 68% at 12 months ($P=0.601$)	41% vs 27% at 12 months ($P<0.001$)	2% vs 4% at 12 months	>99% vs 97% at 12 months	GI- and liver-related events were more frequent with bosutinib, whereas neutropenia, musculoskeletal disorders, and edema were more frequent with imatinib
Ponatinib ^{b,172,173}	EPIC (N=307) Ponatinib 45 mg QD vs imatinib 400 mg QD	74% vs 53% at any time	31% vs 3% at 3 months 62% vs 22% at 6 months 86% vs 33% at 9 months 80% vs 39% at 12 months			More AEs and more serious AEs, including arterial thrombotic events, in the ponatinib arm compared with the imatinib arm; trial suspended early, due to safety concerns

Notes: ^aKaplan–Meier estimate accounting for high rates of crossover and discontinuation; ^bnot approved for newly diagnosed chronic myeloid leukemia.

Abbreviations: AEs, adverse events; BID, twice daily; CCyR, complete cytogenetic response; FISH, fluorescence in situ hybridization; GI, gastrointestinal; MMR, major molecular response; mRNA, messenger RNA; Ph⁺, Philadelphia chromosome–positive; QD, once daily; TKI, tyrosine-kinase inhibitor.

Dasatinib

Dasatinib is an oral BCR–ABL inhibitor that is more potent than imatinib and can inactivate several imatinib-resistant forms of BCR–ABL, with the exception of T315I/A, Q252H, E255K/V, F317L/I, and V299L.^{81,82} Dasatinib also inhibits Src family TKs, which have been shown to contribute to imatinib resistance through the activation of BCR–ABL-independent oncogenic pathways.⁷⁹ In the Phase II START-C trial, dasatinib 70 mg twice daily (BID) resulted in durable hematologic and CyRs in patients (n=387) with CP CML resistant to or intolerant of imatinib. This included CCyR in 49% of patients (40% imatinib resistant and 75% imatinib intolerant) at a median follow-up of 15.2 months.⁸³ Grade 3/4 adverse events (AEs) consisted mainly of myelosuppression, pleural effusion, and dyspnea. A Phase III follow-on study

comparing four dosing regimens of dasatinib in 724 patients found that dasatinib 100 mg once daily (QD) resulted in comparable responses to the 70 mg BID dose and necessitated fewer dose reductions or interruptions.⁸³ Furthermore, the 100 mg QD dose resulted in less toxicity, with significantly lower rates of any grade pleural effusion and grade 3/4 thrombocytopenia. Discontinuation due to toxicity occurred in 4% of patients receiving 100 mg QD versus 11% receiving 70 mg BID. PFS and overall survival rates at 24 months were 80% and 91%, respectively, for 100 mg QD, and 76% and 88%, respectively, for 70 mg BID.⁸⁴ Based on the more favorable benefit–risk profile, the 100 mg QD dose of dasatinib was selected as the starting dose and approved for the treatment of patients with CP CML resistant or intolerant to imatinib.^{83,84}

When dasatinib was compared directly with imatinib in 519 patients with newly diagnosed CP CML enrolled in the Phase III DASISION trial, responses occurred more rapidly and to a greater degree in the dasatinib arm. Significantly more patients treated with dasatinib achieved a CCyR or a major molecular response (MMR) compared with those treated with imatinib by 12 months (Table 6).⁸⁴ Rates of disease progression were also numerically lower for dasatinib. Based on this trial, dasatinib was approved in 2010 as first-line treatment for patients with newly diagnosed CP CML (Table 5).⁸⁵ At the 4-year follow-up, dasatinib continued to demonstrate higher and faster cytogenetic and molecular responses compared with imatinib, with no unexpected toxicity through year 4. However, no long-term differences in overall survival (93% vs 92%) or PFS (90% vs 90%) rates were reported.⁸⁶

Nilotinib

Nilotinib is also an oral inhibitor of BCR–ABL indicated for first- and second-line treatment of patients with CP CML (Table 5).⁸⁷ Nilotinib binds to BCR–ABL more tightly than imatinib, and inhibits most of the clinically relevant mutated forms of *BCR–ABL*, with the exception of T315I, Y253H, E255K/V, and F359V/C.⁸⁸ In a Phase II study of 280 patients with CP CML after imatinib failure or intolerance, nilotinib 400 mg BID resulted in MCyR and CCyR at 6 months in 48% and 31% of patients, respectively.⁸⁹ Nilotinib treatment was well tolerated, with a safety profile that included mainly low-grade dermatologic events and transient grade 3 or 4 myelosuppression. The 48-month estimated rate of overall survival was 78%, and was positively correlated with deeper levels of molecular responses at 3 and 6 months.⁹⁰ Furthermore, long-term results indicated that patients who were in CHR at baseline had significantly higher PFS rates compared with those who did not have responses with initial therapy (71% vs 49%, $P=0.001$).⁹⁰ Nilotinib was also effective in patients with imatinib-resistant or -intolerant accelerated phase CML.⁹¹ The MCyR rate was 29% in this population of patients who had limited therapeutic options, and overall survival was 79% after 12 months of follow-up. The most common grade ≥ 3 AEs were thrombocytopenia, neutropenia, and bilirubin and lipase elevations.

The Phase III ENESTnd trial in patients with newly diagnosed CP CML showed that nilotinib was associated with higher rates of CCyR and MMR at 12 months compared with imatinib (Table 6); these higher response rates were observed even among patients with Sokal high-risk CML.⁹² Furthermore, patients treated with nilotinib at either dose had

significantly lower rates of disease progression compared with imatinib. At the 4-year follow-up, nilotinib treatment remained associated with significantly higher rates of MMR and freedom from progression to accelerated phase or blast phase compared with imatinib; however, overall survival and PFS rates with nilotinib and imatinib were similar.⁹³ A recent analysis from this trial found that nilotinib was associated with a reduced incidence of treatment-emergent *BCR–ABL* mutations compared with imatinib.⁹⁴

Despite the promising responses observed with long-term dasatinib and nilotinib, neither agent has been effective against the T315I mutation, and neither has demonstrated improved overall survival compared with imatinib. Furthermore, some patients develop resistance to or are intolerant of these first-line therapies. Additional therapeutic agents, described herein, have been approved for the treatment of patients with CML after failure of previous therapies and for the treatment of patients with the T315I mutation.

Bosutinib

Bosutinib is a dual BCR–ABL/Src TKI approved for second-line treatment of patients who did not respond to a previous TKI (Table 5).⁹⁵ It is effective against many *BCR–ABL* mutations that are resistant to imatinib, dasatinib, and nilotinib, with the exception of T315I and V299L.^{96,97} In a Phase I/II study of 288 patients with CP CML who were resistant to or intolerant of imatinib therapy, bosutinib treatment was associated with CHR, MCyR, and CCyR rates of 86%, 53%, and 41%, respectively, after a median follow-up of 24.2 months.⁹⁸ Bosutinib treatment was generally well tolerated, with mild-to-moderate transient myelosuppression, gastrointestinal toxicity, and rash. At 4 years, the rate of disease progression to accelerated phase or blast phase was 22% for patients with imatinib resistance and 10% for patients intolerant of imatinib therapy.⁹⁹ In part 2 of this study, a separate cohort of 118 patients previously treated with imatinib followed by dasatinib and/or nilotinib were evaluated for response to bosutinib 500 mg/day.¹⁰⁰ Third- or fourth-line treatment with bosutinib was associated with 73% CHR, 32% MCyR, and 24% CCyR. At a median follow-up of 28.5 months, five patients had progressed to accelerated phase or blast phase.¹⁰⁰ Toxicities were similar to those seen in the patients treated with second-line bosutinib. Gastrointestinal toxicities, including diarrhea, nausea, and vomiting, occurred in 81%, 43%, and 32% of patients, respectively. Although most of these toxicities were grade 1 or 2, they may pose significant challenges for long-term therapy. Bosutinib was also effective in patients with accelerated phase or blast phase CML following imatinib intolerance or resistance,¹⁰¹ leading to its

approval in patients with all three phases of CML who are resistant or intolerant to previous TKI therapy.

Bosutinib was compared with imatinib in the Phase III BELA trial enrolling patients with newly diagnosed CP CML.¹⁰² There was no significant difference between bosutinib and imatinib in terms of CCyR at 12 months (Table 6), and bosutinib was not approved for use in patients with newly diagnosed CP CML.

Ponatinib

Ponatinib is a BCR–ABL inhibitor indicated for use in patients with T315I-positive CML or in whom no other TKI is indicated (Table 5).¹⁰³ The antileukemic property of ponatinib is based on its inhibitory activity of all mutated forms of BCR–ABL, including the T315I mutant, which is resistant to all other currently available BCR–ABL TKIs.¹⁰⁴ The single-arm Phase II PACE trial assessed ponatinib in patients with chronic phase, accelerated phase, or blast phase CML who were resistant to or intolerant of dasatinib or nilotinib or who had the T315I mutation.¹⁰⁵ For patients with CP CML, ponatinib treatment was associated with MCyR in 56%, CCyR in 46%, and MMR in 34% of patients at a median follow-up of 15 months. Responses were durable and occurred regardless of *BCR–ABL* mutation, with no mutations showing resistance to ponatinib. In addition, 57% of patients with accelerated phase and 32% with blast phase CML had major hematologic responses, and 34% and 18%, respectively, had MCyR, leading to ponatinib's approval for treatment of all three phases of CML.¹⁰⁵

Recently, the Phase III EPIC trial was suspended and marketing temporarily halted following an investigation of the drug's safety profile.¹⁰⁶ A report from the US Food and Drug Administration (FDA) found that approximately 24% of patients in the Phase II study and approximately 48% of patients in the Phase I study experienced some degree of cardiovascular AEs after median treatment duration of 1.3 years and 2.7 years, respectively.¹⁰⁷ After implementation of additional recommended safety monitoring, ponatinib is recommended in the NCCN guidelines only for patients who have the T315I mutation or who have not responded to two or more TKIs.¹² The US prescribing information contains a black-box warning regarding vascular occlusion, heart failure, and hepatotoxicity. Of note, rebastinib, a conformational control inhibitor of BCR–ABL1 that has demonstrated strong activity against the T315I mutation in preclinical studies, including inhibition of primary cells from patients with CML,^{108,109} is currently in early clinical development for T315I-positive CP CML (NCT00827138).

Beyond TKIs

Results from TKIs in CML have reinforced the key nature of constitutive BCR–ABL activation in driving the disease. Targeted inhibitors have led to high response rates and very good overall survival. However, point mutations in *BCR–ABL* can lead to acquired resistance in patients who have previously achieved a response. For patients with disease that fails multiple TKIs, other types of therapy are being developed, such as the novel, first-in-class protein-synthesis inhibitor omacetaxine. This approved agent has a unique mechanism of action that is independent of BCR–ABL and effective in patients with the T315I mutation.¹⁰⁵

Ph-negative MPNs

Based on the discovery of the *JAK2*^{V617F} mutation in patients with MPNs, TKIs have been developed to specifically target the JAK–STAT pathway. These have resulted in clinical responses and quality-of-life and survival improvements in many patients. However, because the responses do not appear to be dependent on the presence or absence of a single targetable mutation and because of intra- and inter-disease variability of genetic aberrations among patients with Ph-negative MPNs, combination therapies of JAK inhibitors with other agents may be required to achieve the degree of success that was seen in CML with imatinib and its successors.

Ruxolitinib

Ruxolitinib is an orally administered JAK1/JAK2 inhibitor that modulates intracellular cytokine signaling by binding to the cytoplasmic JAK1 and JAK2 kinases, thus inhibiting their activity. Based on the results of two Phase III studies,^{35,36} ruxolitinib became the first-in-class drug to be approved for the treatment of patients with intermediate- or high-risk MF (Table 5).¹¹⁰ The COMFORT-I and COMFORT-II trials (NCT00952289 and NCT00934544, respectively) demonstrated that ruxolitinib therapy was associated with significant reduction of splenomegaly and improved MF-related symptoms compared with placebo or best-available therapy (BAT).^{35,36} Subsequent follow-up analyses have demonstrated that long-term ruxolitinib treatment provided sustained clinical benefit for up to 3 years.^{111–114} Importantly, this benefit was observed independent of *JAK2*^{V617F} mutational status. In COMFORT-I, mean changes in spleen volume from baseline to week 24 were –34.5% for ruxolitinib-treated patients with the *JAK2*^{V617F} mutation and –23.8% for those without the mutation. Patients in the placebo arm showed increases in spleen volume of 8.1% and 8.4% for those with and without the mutation, respectively.³⁵ Among

patients with $JAK2^{V617F}$ -positive MF in COMFORT-II, spleen volume reduction $\geq 35\%$ by week 48 was observed in 33% of those receiving ruxolitinib versus 0% receiving BAT. The corresponding rates in patients who were $JAK2^{V617F}$ -negative were 14% and 0% for ruxolitinib and BAT, respectively.³⁶ Furthermore, symptom improvement was not dependent on mutational status: the mean change in total symptom score was -52.6% (improvement) in the ruxolitinib arm of COMFORT-I and 42.8% (worsening) in the placebo arm among $JAK2^{V617F}$ -positive patients. Corresponding changes for $JAK2^{V617F}$ -negative patients were -28.1% and $+37.2\%$ in the ruxolitinib and placebo arms, respectively.

In the COMFORT studies, the significant clinical responses observed in patients with the $JAK2^{V617F}$ mutation were generally not accompanied by major allele burden reductions. In COMFORT-I, ruxolitinib treatment was associated with a mean decrease in $JAK2^{V617F}$ allele burden of 10.9% at week 24 and 21.5% at week 48, whereas patients in the placebo arm experienced a mean increase in $JAK2^{V617F}$ allele burden of 3.5% at week 24 and 6.3% at week 48.³⁵ Despite the modest short-term effect on allele burden, ruxolitinib may have disease-modifying properties. Results of survival analyses from the COMFORT studies, including a pooled 3-year survival analysis from both trials,¹¹⁴ suggest that ruxolitinib was associated with a 30%–50% survival advantage compared with placebo or BAT that was consistently observed, despite continuing crossover from the control arms.^{35,36,111–113} In addition, six patients in the ruxolitinib arm of COMFORT-I achieved a complete molecular response after a median treatment duration of 27.5 months, and 20 patients in the study achieved a partial molecular response after a median treatment duration of 22.2 months.¹¹⁵

Anemia and thrombocytopenia were the most common AEs associated with ruxolitinib treatment in the COMFORT trials, typically observed in the first 8–12 weeks of treatment. These events were usually manageable with dose modification or brief treatment interruption, and rarely led to discontinuation.^{35,36,111–114}

Based on the results of the randomized, open-label, multicenter Phase III RESPONSE trial,¹¹⁶ ruxolitinib was also recently approved by the FDA for the treatment of patients with PV who have had an inadequate response to or are intolerant of hydroxyurea (Table 5). RESPONSE enrolled 222 patients and compared the proportion of patients with both hematocrit control (one or more instance of phlebotomy eligibility during the first 8 weeks after randomization and no phlebotomy eligibility afterwards) and a $\geq 35\%$ reduction in spleen volume from baseline to week 32 for those randomized

to ruxolitinib versus those randomized to standard therapy. This composite primary end point was achieved by 20.9% and 0.9% of patients receiving ruxolitinib and standard therapy, respectively ($P < 0.001$). Overall, 60%, 38%, and 49% in the ruxolitinib arm compared with 20%, 1%, and 5% in the standard therapy arm achieved hematocrit control, a $\geq 35\%$ reduction in spleen volume, and a $\geq 50\%$ reduction in total symptom score, respectively. Safety and tolerability profiles were similar for ruxolitinib and standard therapy. Nonhematologic AEs were mostly grade 1 or 2, and no grade 3 or 4 anemia was reported.¹¹⁶ Recent follow-up data from RESPONSE for a median exposure to ruxolitinib of 111 weeks showed that ruxolitinib provided durable and comprehensive hematologic control of hematocrit, white blood cell count, and platelet count.¹¹⁷

Preliminary results from an open-label, single-arm, multicenter Phase II study further suggest that combination therapy with $IFN\alpha_2$ and ruxolitinib may be effective in eliciting both hematologic responses and substantial reductions in $JAK2^{V617F}$ allele burden in patients with PV.¹¹⁸ Of the 20 patients with PV with available data, 15 (75%) achieved a complete response and five (25%) achieved a partial response during the first 3 months of therapy.¹¹⁸

Momelotinib

Momelotinib is a JAK1/JAK2 inhibitor currently in Phase III development for MF (SIMPLIFY 1, NCT01969838). A Phase I/II trial and subsequent long-term follow-up and extension study in patients with high- or intermediate-risk primary or post-PV/ET MF showed that the majority of evaluable patients treated with momelotinib had durable spleen response and symptom improvement, with a median treatment duration of 507 days.¹¹⁹ Momelotinib treatment was generally well tolerated, with thrombocytopenia, dizziness, and peripheral neuropathy being the main treatment-emergent AEs.^{119–121} Peripheral neuropathy generally manifested as grade 1 sensory symptoms in the feet and/or hands, and appeared to involve length-dependent sensorimotor large- and small-fiber neuropathy with axonal features.¹²¹ In contrast, Wernicke's encephalopathy, a serious neurologic condition caused by thiamine deficiency, has been associated only with the JAK2 inhibitor fedratinib.^{122,123} Consequently, clinical development of fedratinib was discontinued, despite the drug's demonstrated efficacy in advanced MF in a large Phase III clinical trial.¹²² In 100 consecutive patients with MF treated with momelotinib at the Mayo Clinic, presence of *CALR* mutations (hazard ratio 0.2, 95% confidence interval 0.04–0.6) and absence of *ASXL1* mutations (hazard ratio

0.3, 95% confidence interval 0.1–0.8) were independently associated with better spleen response (as per International Working Group-Myeloproliferative Neoplasms Research and Treatment) in a multivariable analysis.¹²⁴

Pacritinib

An in vitro kinome screening analysis of pacritinib (SB1518), a dual JAK2–FLT3 inhibitor,¹²⁵ indicated that it inhibits both *JAK2*^{V617F} and wild-type *JAK2* at similar concentrations.¹²⁶ Results of Phase II studies in patients with MF suggested that pacritinib treatment may provide spleen volume reduction and clinically significant improvement in symptom burden with minimal myelosuppression, including in patients with low baseline platelet counts.^{127,128} Recent results from PERSIST-1 (NCT01773187), a randomized controlled study of pacritinib versus BAT in patients with moderate or severe MF and any degree of anemia or thrombocytopenia (N=327), showed that the primary end point of $\geq 35\%$ decrease in spleen volume at week 24 was achieved by 19.1% of patients randomized to pacritinib versus 4.7% of patients randomized to BAT ($P=0.0003$).¹²⁹ The corresponding proportions among patients with a platelet count $< 50 \times 10^9/L$ (15% of the total study population) were 22.9% versus 0%, demonstrating that pacritinib can provide clinically significant improvements in splenomegaly in patients with severe thrombocytopenia. Generally low-grade gastrointestinal disturbances were the most common AEs.¹²⁹

Agents in early stages of clinical development

Additional TKIs are being investigated in Phase I and Phase II studies in patients with Ph-negative MPNs. These include gandotinib (LY2784544),¹³⁰ a *JAK2*^{V617F}-selective inhibitor; INCB39110,¹³¹ a JAK1-selective inhibitor; and the JAK2-selective inhibitor NS-018.¹³²

Beyond TKIs

Overall, although the original rationale behind the development of TKIs for classic Ph-negative MPNs was based on the identification of the *JAK2*^{V617F} mutation, clinical evidence suggests that JAK inhibitors can be effective regardless of *JAK2*^{V617F} mutational status. Based on the complex genetic profile of classic Ph-negative MPNs, multitargeted therapies that inhibit multiple oncogenic pathways are likely to provide superior clinical benefit while reducing the possibility of the activation of mechanisms of resistance.¹³³ For example, additional compounds in clinical development for the treatment of MF include the non-TKIs imetelstat,¹³⁴ pomalidomide,^{135–137} panobinostat,¹³⁸ and PRM-151,¹³⁹ and

some of these agents may prove to be useful in combination with JAK inhibitors.

Nonclassic Ph-negative MPNs

Because of the rarity of the nonclassic Ph-negative MPNs, clinical trial evidence is scarce for these diseases. Case reports indicate that treatment has drawn from knowledge of TKIs in other MPNs.

Imatinib

In a case study of a PDGFRA/B-negative CEL-NOS patient, imatinib treatment was associated with rapid eosinophil clearance, complete response, including normalization of bone marrow histomorphology, and improvement of disease-related symptoms.¹⁴⁰ Other institutions have had limited success with imatinib in CEL-NOS, a rare and aggressive disease that does not respond well to conventional treatment.¹⁴¹

Imatinib has also been used to treat systemic mastocytosis, as have dasatinib, nilotinib, masitinib, and midostaurin, all of which can block activated KIT TK. However, the clinical efficacy of TKIs in patients with kinase-domain *KIT*^{D816V} mutations remains unclear.^{50,142,143} Further studies are needed to determine whether TKIs have a clinical advantage over cladribine or IFN α in mastocytosis.

Ruxolitinib

In preclinical studies, chronic myelomonocytic leukemia cells have demonstrated dependence on granulocyte macrophage colony-stimulating factor through activation of the JAK–STAT pathway.¹⁴⁴ Based on these observations, the efficacy and safety of ruxolitinib are currently being investigated in a Phase I/II study in patients with chronic myelomonocytic leukemia.

In patients with CNL and aCML, the discovery of different classes of *CSF3R* mutations has provided the rationale for the investigation of JAK or Src inhibitors to target membrane proximal or truncation *CSF3R* mutations, respectively.⁴⁸ Consistent with preclinical observations showing that ruxolitinib treatment inhibited colony formation of cells with *CSF3R* membrane-proximal mutations,⁴⁸ ruxolitinib has been shown to provide hematologic responses in individual patients with CNL or aCML who had the JAK-activating membrane-proximal *CSF3R*^{T618I} mutation.^{48,145–147} Dasatinib may be useful in patients with CNL who do not respond to ruxolitinib, based on preclinical data demonstrating that dasatinib inhibited colony formation of primary cells from patients with Src-activating *CSF3R* truncation mutations that were insensitive to ruxolitinib treatment.⁴⁸

The use of TKIs in nonclassic Ph-negative MPNs may be a feasible approach for some patients harboring TK-activating mutations; however, the bulk of evidence for these rarer diseases has been obtained from small case studies and will need to be validated in larger clinical trials.

Treatment goals and expectations in CML and MF

Prior to the introduction of BCR–ABL-targeted TKI therapy, survival for patients with CML was poor. Based on the results of the imatinib trials, which reported unprecedented hematologic responses and CyRs, and mindful of the prognostic implications of these studies, CHR became the first treatment goal, as failure to achieve CHR by 3 months constituted failure as per European LeukemiaNet and NCCN criteria.^{12,148} As TKIs became more specific, more stringent CyR and molecular responses became the goals of therapy.¹⁴⁹ CyR is the strongest predictor of outcome in patients with CML receiving TKIs, as achieving an early CCyR was associated with significantly better event-free and overall survival compared with patients who did not achieve CCyR.¹⁵⁰ Once CCyR is achieved, more sensitive assays are used to quantify the presence of residual CML cells by PCR analysis of *BCR–ABL* messenger RNA expression levels. Achievement of MMR is associated with very low odds of progression to advanced disease and superior PFS compared with patients who do not achieve MMR,¹⁵¹ even among patients receiving second-line therapy after imatinib failure.¹⁵² Responses are assessed every 3 months, and if transcript levels of *BCR–ABL* increase, patients should be analyzed for mutations, which can help guide treatment decisions.⁴ Since the introduction of imatinib in 2001, survival has improved significantly in patients with CML, especially those with CP or accelerated phase disease.¹⁴ In addition, imatinib therapy minimized the impact of previous prognostic factors and Sokal risk on survival.

Although definitions of complete and partial cytogenetic or molecular remission were recently introduced for the evaluation of treatment efficacy in patients with MF, response in these patients remains essentially defined by clinical criteria, with complete and partial remission based on the degree of normalization of bone marrow findings and hematologic laboratory results, and resolution of splenomegaly and symptoms. In addition, to qualify as response, any clinical benefits have to last at least 12 weeks.¹⁵³ Hematologic and clinical responses, such as spleen size reduction and symptom improvement, are not dependent on the presence of a specific mutation, and JAK-inhibitor therapy is able to provide rapid and durable clinical benefits in MPNs

that have not been seen with conventional therapies. Most importantly, in addition to providing clinically significant reductions in splenomegaly and symptom burden in the majority of patients with MF, the JAK1/JAK2 inhibitor ruxolitinib is the first and only therapy in MF to be associated with survival advantages compared with no or standard therapy.^{111,113,114,154} Furthermore, long-term therapy with ruxolitinib was shown to result in gradual decreases in mutant allele burden, and in some cases complete molecular and/or histologic responses.^{115,155–157} Together, these observations are evidence of the ability of ruxolitinib to modify the natural history of MF.

Conclusion

There are profound differences between the mechanisms of disease in CML and MF. While the success of BCR–ABL-targeting TKIs in CML is contingent on the presence of the Ph chromosome and the *BCR–ABL* mutation, the clinical efficacy of JAK inhibitors in MF is independent of *JAK2*-mutational status, and has demonstrated positive results in patients with both wild-type and mutant *JAK2*. The oncogenic BCR–ABL TK is the driving event for CML initiation and progression, and is the molecular signature for diagnosis and response to treatment. TKIs, such as imatinib, dasatinib, nilotinib, bosutinib, and ponatinib, target this aberrant fusion protein kinase and some of its acquired mutated forms, and have demonstrated significant complete hematologic, cytogenetic, and molecular responses in patients with newly diagnosed or advanced CML.

In contrast, the molecular pathobiology of Ph-negative MPNs is complex, involving multiple mutations and multiple pathways. Unlike CML, classic Ph-negative MPNs have no singular disease signature mutation, but instead have a preponderance of mutations that produce disease initiation and progression. The identification of mutations affecting *JAK2* leading to aberrant JAK–STAT signaling in patients with MPNs has propelled the development of JAK inhibitors, such as ruxolitinib, which in controlled clinical trials have shown successful control of disease-related symptoms, improvement in quality-of-life measures, and overall survival benefit in patients with MF. However, contrary to initial expectations, the clinical efficacy of JAK inhibitors in patients with Ph-negative MPNs is not dependent on the presence of any one specific mutation as a driver of the disease or on the reduction of *JAK2*^{V617F} allele burden in patients who carry this mutation. Current evidence suggests that overactive JAK–STAT signaling is a central defect in Ph-negative MPNs that extends beyond the neoplastic clones carrying the somatic mutation, and is independent of the specific mutation background. By

providing rapid and durable clinical benefit with concomitant quality-of-life improvement for patients with MF, JAK inhibitors constitute significant therapeutic progress over historically limited treatment options with at best transient benefits. In the near future, it is probable that novel combinations of targeted therapies that complement JAK inhibition will further improve the treatment landscape for classic Ph-negative MPNs by addressing additional components of the complex pathobiology of these diseases.

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Author contributions

All authors contributed to the conception of this review, critically reviewed and revised each draft for intellectual content, and approved the final version of the manuscript for publication.

Disclosure

JPI is an investigator for Novartis and Ariad and served as a consultant/advisor for Novartis, Bristol-Myers Squibb, and Ariad. KLS has received research funding from Incyte Corporation, is an adviser for Novartis and Ariad, and serves on the speaker bureau for Novartis, Ariad, and Pfizer. GMCY reports no conflict of interest in this work. RSK serves as a consultant/advisor and member of the speaker bureau for Incyte Corporation and Novartis, is a consultant/advisor for CTI Biopharma Corp and Celgene, and has received research funding from Celgene and GlaxoSmithKline. The authors report no other conflicts of interest in this work.

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