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

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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...
(CEL-NOS), mast-cell disease (mastocytosis), and MPNs unclassifiable. 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 $BCR-ABL$. $BCR-ABL$ positivity is exclusively associated with CML, whereas Ph-negative MPNs are a heterogeneous group that includes all other MPNs.

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 marrow and involving one or all myeloid lineages. 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. The molecular consequence of this t(9;22) translocation is the creation of $BCR-ABL$, a chimeric protein with constitutive oncogenic TK activity. Expression of $BCR-ABL$ results in the upregulation of multiple downstream oncogenic pathways, and has been shown to be the initiating event for the leukemogenesis of CML. 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 $\beta$-catenin.

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. 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. 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.

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. 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. 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 MF differ regarding life expectancy at diagnosis, risk of leukemic transformation, 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. Patients with PV and ET have an approximate 10%–20% probability of evolving to post-PV MF or post-ET MF. In recognition of the largely indistinguishable clinical manifestations, clinical course, and histopathological features of PMF, post-PV MF, and post-ET MF, 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. 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.

Unlike CML, classic Ph-negative MPNs are characterized by complex and variable mutation profiles and
clonal evolution with unknown disease-initiating event(s) (Figure 1). 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.

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 MPN$^{31}$ 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

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.$^{39}$
Abbreviation: TPO, thrombopoietin.
in JAK2 exon 12,34,38 and patients with PMF or ET may have mutations in the thrombopoietin-receptor gene MPL39 or in the CALR gene. With few exceptions, CALR, JAK2, and MPL mutations appear to be mutually exclusive within the same patient.34,26,40,47 Although the precise mechanism of action of the CALR mutation remains unclear, evidence from an integrated genomic analysis38 and preclinical studies26,41 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 JAK2V617F-negative MPN.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 JAK2V617F 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 shortened 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,

<table>
<thead>
<tr>
<th>Table 1 Common mutations in classic Ph-negative MPNs</th>
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<tr>
<td>Mutations affecting JAK–STAT signaling</td>
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<tr>
<td>JAK2V617F</td>
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<tr>
<td>JAK2 exon 12</td>
</tr>
<tr>
<td>MPL exon 10</td>
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<tr>
<td>CALR exon 9</td>
</tr>
</tbody>
</table>

**Somatic mutations in epigenetic modifiers**

- **TET2**, any of 12 exons26,34,162
  - PV: -16%
  - ET: -5%
  - PMF: -17%
  - Post-PV MF: -14%
  - Post-ET MF: -14%
- **ASXL1** exon 1224,32,24,164
  - PV: -7%
  - ET: -4%
  - MF: -20%
- **IDH1/IDH2** exon 429,34,165
  - PV: -2%
  - ET: -1%
  - PMF: -4%
  - Post-PV MF: -1%
  - Post-ET MF: -1%
- **EZH2** mutations occur across the gene’s 20 exons; intronic mutations have been reported26,167
  - PMF: -6%
  - Post-PV MF: -1%
  - Post-ET MF: -9%
- **DNMT3A** methyltransferase domain40,168,169
  - PV: -7%
  - ET: -3%
  - MF: -15%

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

- 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 JAK2V617F

• Higher risk of postdiagnosis arterial thrombosis in ET
• Lower hemoglobin level and higher risk of transfusion dependence in MF
• 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

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency of mutation</th>
<th>Prognostic relevance</th>
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<tbody>
<tr>
<td>JAK2V617F</td>
<td>32,34,13,199</td>
<td>• Potentially higher risk of thrombotic and vascular complications in ET</td>
</tr>
<tr>
<td>JAK2 exon 12</td>
<td>34,38,160</td>
<td>• Increased risk of fibrotic transformation in ET and PV</td>
</tr>
<tr>
<td>MPL exon 10</td>
<td>34,39,161,162</td>
<td>• Leukocytosis and larger splenomegaly in PV, ET, and PMF</td>
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<td>CALR exon 9</td>
<td>35,40,41,46</td>
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“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).1,12 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 ≥15% blasts, ≥30% blasts plus promyelocytes, ≥20% basophils in blood or bone marrow, platelets <100 × 109/L unrelated to therapy, or cytogenetic clonal evolution; and the blast phase as ≥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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Major criteria</th>
<th>Minor criteria</th>
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<tbody>
<tr>
<td>Ph-positive classic MPNs</td>
<td></td>
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<tr>
<td>Chronic myeloid leukemia</td>
<td>• Positive for BCR–ABL1</td>
<td>• Constitutional symptoms:</td>
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<tr>
<td></td>
<td></td>
<td>• Fatigue</td>
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<td>• Weight loss</td>
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<td></td>
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<td>• Night sweats</td>
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<td></td>
<td></td>
<td>• Splenomegaly</td>
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<tr>
<td></td>
<td></td>
<td>• Anemia</td>
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<tr>
<td></td>
<td></td>
<td>• Leukocytosis (median WBC count ~100×10⁹/L; range 12–1,000×10⁹/L)</td>
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<td></td>
<td></td>
<td>• Neutrophils in different stages of maturation, with predominant segmented neutrophils and myelocytes</td>
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<td></td>
<td></td>
<td>• Presence of absolute basophilia</td>
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<td></td>
<td></td>
<td>• Normal or elevated platelet count (rare thrombocytopenia)</td>
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<td></td>
<td></td>
<td>• BM granulocytic proliferation, leading to increased cellularity</td>
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<td></td>
<td></td>
<td>• Blasts &lt;5% of nucleated BM cells</td>
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<td></td>
<td></td>
<td>• BM erythroid islands reduced in number and size</td>
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<td></td>
<td></td>
<td>• Smaller and megakaryocytes with hypolobated nuclei (dwarf megakaryocytes) in BM</td>
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<tr>
<td></td>
<td></td>
<td>• Moderate or marked reticulin fibrosis in BM</td>
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<tr>
<td>Ph-negative classic MPNs</td>
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<tr>
<td>PMF</td>
<td>• Megakaryocyte proliferation and atypia accompanied by either reticulin and/or collagen fibrosis</td>
<td>• Leukoerythroblastosis</td>
</tr>
<tr>
<td></td>
<td>• Not meeting WHO criteria for CML, PV, MDS, or other myeloid neoplasm</td>
<td>• Increased serum LDH level</td>
</tr>
<tr>
<td></td>
<td>• Demonstration of JAK2 V617F or other clonal marker or no evidence of reactive marrow fibrosis</td>
<td>• Anemia</td>
</tr>
<tr>
<td>PV</td>
<td>• Hb &gt; 18.5 g/dL (men), &gt; 16.5 g/dL</td>
<td>• Palpable splenomegaly</td>
</tr>
<tr>
<td></td>
<td>• Presence of JAK2 V617F or JAK2 exon 12 mutation</td>
<td>• BM trilineage myeloproliferation</td>
</tr>
<tr>
<td>Essential thrombocytethemia</td>
<td></td>
<td>• Subnormal serum erythropoietin level</td>
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<td></td>
<td>• EEC growth</td>
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<tr>
<td>Ph-negative nonclassic MPNs</td>
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<tr>
<td>Systemic mastocytosis</td>
<td>• Multifocal, dense infiltrates of mast cells (≥15 mast cells in aggregates) detected in sections of BM and/or other extracutaneous organ(s)</td>
<td>• In biopsy sections of BM or other extracutaneous organs, &gt;25% of the mast cells in the infiltrate are spindled-shaped or have atypical morphology, or of all mast cells in BM aspirate smears, &gt;25% are immature or atypical</td>
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<td>• Detection of an activating mutation point at codon 816 of KIT in BM, blood, or another extracutaneous organ</td>
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<td></td>
<td>• Mast cells in BM, blood, or other extracutaneous organs express CD2 and/or CD2S, in addition to normal mast-cell markers</td>
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<tr>
<td></td>
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<td>• Serum total tryptase persistently &gt; 20 ng/mL (parameter valid only in the absence of an associated clonal myeloid disorder)</td>
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</table>
Peripheral blood leukocytosis (WBC count ≥25×10^9/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 PDGFRα, PDGFRβ, or FGFR1

No evidence of PV, ET, PMF, MDS, or MDS/MPN

No granulocytic dysplasia or myelodysplastic changes in other myeloid lineages

Monocytes <1×10^9/L

Eosinophils ≥1.5×10^9/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 PDGFRβ

No FIP1L1–PDGFRα fusion gene or any other rearrangement of PDGFRα

No rearrangement of FGFR1

Blast count in the peripheral blood and BM <20%

No inv(16)(p13.1q22) 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: ‘Diagnosis of PMF requires meeting all three major criteria and two minor criteria; ‘diagnosis of PV requires meeting either both major criteria and one minor criterion or the first major criterion and two minor criteria; ‘diagnosis of ET requires meeting all four major criteria; ‘small-to-large megakaryocytes with aberrant nuclear:cytoplasmic ratio, hyperchromatic and irregularly folded nuclei, and dense clustering. In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis (ie, prefibrotic PMF). Hb or hematocrit >99th 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; ‘diagnosis 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; EE C, 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. 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 polycythemias in patients with JAK2 mutation-negative PV and ET in patients with masked PV.

**Nonclassic Ph-negative MPNs**

Diagnosis of nonclassic MPNs can be difficult, and generally requires absence of BCR–ABL, dyserythropoiesis, and granulocyte dysplasia. 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. 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. 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).

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. In addition to these categories, the Hasford system also includes percentage of basophils and eosinophils. 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. 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. 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.

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. 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. 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. Currently, only the Sokal and Hasford scoring systems are recommended by the National Comprehensive Cancer Network (NCCN) guidelines. 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.

**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). 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 >25×10^9/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. 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 <100×10^9/L, transfusion dependence, and unfavorable karyotype during disease course. Additionally, unfavorable karyotype and platelet
count <100×10^9/L have been demonstrated to be independent predictors of leukemia-free survival and leukemic transformation. 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. 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. Despite disease similarities between de novo and transformed MF and the common use of prophylactic 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. A separate scoring system may be warranted to aid physicians in treatment of these patients. In addition, recent studies suggest that the specific mutation profiles may have a significant effect on prognosis in patients with PMF.

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

<table>
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<tr>
<th>Table 3 Risk stratification systems for chronic myeloid leukemia</th>
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<tbody>
<tr>
<td><strong>Sokal</strong></td>
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<tr>
<td>Year introduced</td>
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<tr>
<td>Predominant treatment modality</td>
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<tr>
<td>Factors</td>
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<td>Risk calculation</td>
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<td>Risk groups</td>
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Note: An intermediate-risk category was not defined for EUTOS.

<table>
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<th>Table 4 Risk stratification for patients with classic Ph-negative myeloproliferative neoplasms</th>
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<tbody>
<tr>
<td><strong>ET</strong></td>
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<tr>
<td>Factors</td>
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<td>Risk group</td>
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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. 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). Patients with PV are categorized as high- or low-risk based on age, leukocyte count, and history of previous thrombosis. 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.

**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 IFNα therapy, and was subsequently approved in 2002 for newly diagnosed CP CML (Table 5). Imatinib was compared with standard IFNα plus low-dose cytarabine in the Phase III IRIS study of 1,106 patients with newly diagnosed CP CML. At the 18-month follow-up, imatinib was superior to IFNα 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.

Despite these promising results, an estimated 20%–30% of patients who achieve CHR and/or CCyR eventually develop secondary (acquired) resistance to imatinib. 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. 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. Second- and third-generation TKIs, such as dasatinib, nilotinib, bosutinib, and ponatinib were designed to overcome imatinib resistance.

<table>
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<tr>
<th>Drug</th>
<th>Mechanism of action</th>
<th>US indications for MPNs</th>
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<tbody>
<tr>
<td>Imatinib</td>
<td>BCR–ABL inhibitor</td>
<td>• Newly diagnosed adult and pediatric patients with Ph+ CML in chronic phase</td>
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<tr>
<td></td>
<td></td>
<td>• Patients with Ph+ CML in blast crisis, accelerated phase, or in chronic phase after failure of IFNα therapy</td>
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<tr>
<td>Dasatinib</td>
<td>BCR–ABL inhibitor, including imatinib-resistant forms</td>
<td>• Newly diagnosed adults with Ph+ CML in chronic phase</td>
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<td></td>
<td></td>
<td>• Adults with chronic, accelerated, or myeloid or lymphoid blast phase Ph+ CML with resistance or intolerance to prior therapy, including imatinib</td>
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<tr>
<td>Nilotinib</td>
<td>BCR–ABL inhibitor, preferentially mutant forms</td>
<td>• Newly diagnosed adult patients with Ph+ CML in chronic phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Chronic phase and accelerated phase Ph+ CML in adult patients resistant to or intolerant to prior therapy that included imatinib</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>Dual BCR–ABL/Src inhibitor, including mutant BCR–ABL resistant to other TKIs</td>
<td>• Adult patients with chronic phase, accelerated phase, or blast phase Ph+ CML with resistance or intolerance to prior therapy</td>
</tr>
<tr>
<td>Ponatinib</td>
<td>Inhibitor of BCR–ABL including T315I-mutated BCR–ABL</td>
<td>• Adult patients with T315I-positive CML (chronic phase, accelerated phase, or blast phase)</td>
</tr>
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<td></td>
<td></td>
<td>• Adult patients with chronic phase, accelerated phase, or blast phase CML for whom no other TKI therapy is indicated</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>JAK1/JAK2 inhibitor</td>
<td>• Patients with intermediate- or high-risk myelofibrosis, including primary myelofibrosis, post-polycythemia vera myelofibrosis and post-essential thrombocythemia myelofibrosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Patients with polycythemia vera who have had an inadequate response to or are intolerant of hydroxyurea</td>
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**Table 5** Approved therapies and their indication for patients with MPNs in the US

**Abbreviations**: CML, chronic myeloid leukemia; MPNs, myeloproliferative neoplasms; Ph+, Philadelphia chromosome–positive; TKIs, tyrosine-kinase inhibitors.
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. Dasatinib also inhibits Src family TKs, which have been shown to contribute to imatinib resistance through the activation of BCR–ABL-independent oncogenic pathways. 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. 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. Furthermore, the 100 mg QD dose resulted in less toxicity, with significantly lower rates of any grade neutropenia and thrombocytopenia. Discontinuation due to toxicity occurred in 4% of patients receiving 100 mg QD versus 11% receiving 70 mg BID in the imatinib arm; trial suspended in the ponatinib arm compared with 91.5% and 96.7% estimated overall survival at 12 months; grade 3/4 AEs that were more common in the combination group included fatigue, depression, myalgia, arthralgia, neutropenia, and thrombocytopenia. Similar rates, mostly grade 1 or 2; nausea, vomiting, fluid retention, and superficial edema more common with imatinib; pleural effusion only reported with dasatinib. Dermatologic events and headache were more common with nilotinib; GI events and fluid retention more common with imatinib. 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.

**Dasatinib**

<table>
<thead>
<tr>
<th>TKI</th>
<th>Study</th>
<th>CCyR (0 Ph cells measured by either conventional or FISH cytogenetic testing)</th>
<th>MMR (≥3-log reduction of BCR–ABL mRNA)</th>
<th>Progression to blast crisis</th>
<th>Kaplan–Meier estimated overall survival</th>
<th>Safety</th>
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<tr>
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<td>Freedom from progression:</td>
<td>97.2% vs 95.1% at 12 months</td>
<td>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.</td>
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<td>96.7% vs 91.5% (P&lt;0.001) at 18 months</td>
<td>97% vs 99% at 12 months</td>
<td>Similar rates, mostly grade 1 or 2; nausea, vomiting, fluid retention, and superficial edema more common with imatinib; pleural effusion only reported with dasatinib.</td>
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<td>46% vs 28% (P&lt;0.001) at 12 months</td>
<td>&lt;1% and &lt;1% at 12 months; Not reported</td>
<td>Dermatologic events and headache more common with nilotinib; GI events and fluid retention more common with imatinib.</td>
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<td>44% and 43% vs 22% (P&lt;0.001) at 12 months</td>
<td>94.3% and 96.7% at 12 months</td>
<td>GI- and liver-related events were more frequent with bosutinib, whereas neutropenia, musculoskeletal disorders, and edema were more frequent with imatinib.</td>
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<td>2% vs 4% at 12 months</td>
<td>&gt;99% vs 97% at 12 months</td>
<td>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.</td>
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<td>31% vs 3% at 3 months</td>
<td>62% vs 22% at 6 months</td>
<td>Freedom from progression; 96.7% vs 91.5% (P&lt;0.001) at 18 months</td>
</tr>
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<td>86% vs 35% at 9 months</td>
<td>80% vs 39% at 12 months</td>
<td>To be determined.</td>
</tr>
</tbody>
</table>

**Notes:** Kaplan–Meier estimate accounting for high rates of crossover and discontinuation; *not 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.
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). 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). 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.

**Nilotinib**

Nilotinib is also an oral inhibitor of BCR–ABL indicated for first- and second-line treatment of patients with CP CML (Table 5). 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. 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. 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. 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). Nilotinib was also effective in patients with imatinib-resistant or -intolerant accelerated phase CML. 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. 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. A recent analysis from this trial found that nilotinib was associated with a reduced incidence of treatment-emergent BCR–ABL mutations compared with imatinib.

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). It is effective against many BCR–ABL mutations that are resistant to imatinib, dasatinib, and nilotinib, with the exception of T315I and V299L. 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. 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. 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. 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. 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, 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. 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). 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. 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. 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.

Recently, the Phase III EPIC trial was suspended and marketing temporarily halted following an investigation of the drug’s safety profile. 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. 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. 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, is currently in early clinical development for T315I-positive CP CML.

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.

Ph-negative MPNs
Based on the discovery of the JAK2V617F 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, ruxolitinib became the first-in-class drug to be approved for the treatment of patients with intermediate- or high-risk MF (Table 5). 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). Subsequent follow-up analyses have demonstrated that long-term ruxolitinib treatment provided sustained clinical benefit for up to 3 years. Importantly, this benefit was observed independent of JAK2V617F mutational status. In COMFORT-I, mean changes in spleen volume from baseline to week 24 were −34.5% for ruxolitinib-treated patients with the JAK2V617F 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.
patients with JAK2V617F-positive MF in COMFORT-II, spleen volume reduction ≥35% by week 48 was observed in 33% of those receiving ruxolitinib versus 0% receiving BAT. The corresponding rates in patients who were JAK2V617F-negative were 14% and 0% for ruxolitinib and BAT, respectively. 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 JAK2V617F-positive patients. Corresponding changes for JAK2V617F-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 JAK2V617F mutation were generally not accompanied by major allele burden reductions. In COMFORT-I, ruxolitinib treatment was associated with a mean decrease in JAK2V617F 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 JAK2V617F allele burden of 3.5% at week 24 and 6.3% at week 48. 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, 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. 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.

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.

Based on the results of the randomized, open-label, multicenter Phase III RESPONSE trial, 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 ≥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 ≥35% reduction in spleen volume, and a ≥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. 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.

Preliminary results from an open-label, single-arm, multicenter Phase II study further suggest that combination therapy with IFN and ruxolitinib may be effective in eliciting both hematologic responses and substantial reductions in JAK2V617F allele burden in patients with PV. 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.

Momelotinib

Momelotinib is a JAK1/JAK2 inhibitor currently in Phase III development for MF (SIMPLIFY I, 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. Momelotinib treatment was generally well tolerated, with thrombocytopenia, dizziness, and peripheral neuropathy being the main treatment-emergent AEs. 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. In contrast, Wernicke’s encephalopathy, a serious neurologic condition caused by thiamine deficiency, has been associated only with the JAK2 inhibitor fedratinib. Consequently, clinical development of fedratinib was discontinued, despite the drug’s demonstrated efficacy in advanced MF in a large Phase III clinical trial. 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 JAK2V617F 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 ≥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 <50×10^9/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.130

**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 ganotinib (LY2784544),130 a JAK2V617F-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 JAK2V617F mutation, clinical evidence suggests that JAK inhibitors can be effective regardless of JAK2V617F mutational status. Based on the complex genetic profile of classic Ph-negative MPNs, multtargeted 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 IFNα 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,49 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. As TKIs became more specific, more stringent CyR and molecular responses became the goals of therapy. 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. 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, even among patients receiving second-line therapy after imatinib failure. 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. Since the introduction of imatinib in 2001, survival has improved significantly in patients with CML, especially those with CP or accelerated phase disease. 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. 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. 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. 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 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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