JAK2 MUTATION AND TREATMENT OF JAK2 INHIBITORS IN PHILADELPHIA CHROMOSOME-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

Susanna Hilda Hutajulu1, Johan Kurnianda2

Division of Hematology and Medical Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Gadjah Mada/Dr Sardjito General Hospital, Yogyakarta, Indonesia

ABSTRACT

The Philadelphia chromosome-negative (Ph-negative) myeloproliferative neoplasms (MPNs) polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) are clonal disorders of multipotent haematopoietic progenitors. The genetic cause of these disorders was not fully defined until a somatic activating mutation in the JAK2 non-receptor tyrosine kinase, JAK2V617F, was identified in most patients with PV and significant proportion of patients with ET and PMF. The discovery of JAK2 mutation has changed the molecular reclassification of MPNs. This also provided a genomic target for therapeutical approach. A number of JAK2 inhibitors have been developed and tested for MPNs. Several JAK2 inhibitors have reached the stage of clinical trial and included patients with intermediate-risk or high-risk MF. This population of MF is best candidate for trials because currently it has no effective therapy and the survival is significantly poor. Considering all clinical data on Ph-negative MPNs, JAK2 inhibitors give a clinical benefit of spleen reduction in approximately 40-50% of patients and abolished symptoms in vast majority of MF cases. The most developed among JAK2 inhibitors is Ruxolitinib, which has demonstrated clinical improvement with well tolerated toxicities. However, JAK2 inhibitor was equally active in patients with and without JAK2 mutation. Other JAK2 inhibitors are less developed but showed a similar clinical benefit. The effect on the natural course of MF in treated patients needs to be further investigated.

INTRODUCTION

Myeloproliferative disorders (MPD), recently renamed as myeloproliferative neoplasms (MPNs), are clonal disorders of transformed multipotent hematopoietic stem cells which manifest clinically by uncontrolled myeloid proliferation. These proliferative syndromes include chronic myeloid leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF), as well as chronic eosinophilic leukaemia (CEL), chronic myelomonocytic leukaemia (CMML), and systemic mastocytosis (SM) and others. Although each of the MPN is identified as a distinct clinicopathological existence, these disorders share common features that discriminate them from other myeloid malignancies such as myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). These features include biology and clinical characteristics. Biologically, MPNs involve a multipotent hematopoietic stem cell with a dominance of the transformed clone over nontransformed progenitors and hypercellularity of the marrow, with apparently unstimulated overproduction of one or more of the formed elements of blood. Clinically, they have an increased risk of thrombosis and bleeding and spontaneous transformation to acute leukemia and marrow fibrosis.

Detection of mutant alleles in CML, CMML, CEL and SM, led to observations that constitutive activation of tyrosine kinase signalling was induced by the mutation. A clear example of these mutated kinases is the product of the Philadelphia (Ph) chromosome, the BCR (breakpoint cluster region)–ABL (Abelson murine leukemia) fusion tyrosine kinase in CML. Accumulating data afterward demonstrated that tyrosine kinase signalling activation was induced by the mutation. A clear example of these mutated kinases is the product of the Philadelphia (Ph) chromosome, the BCR (breakpoint cluster region)–ABL (Abelson murine leukemia) fusion tyrosine kinase in CML. Accumulating data afterward demonstrated that tyrosine kinase signalling activation was induced by the mutation. A clear example of these mutated kinases is the product of the Philadelphia (Ph) chromosome, the BCR (breakpoint cluster region)–ABL (Abelson murine leukemia) fusion tyrosine kinase in CML. Accumulating data afterward demonstrated that tyrosine kinase signalling activation was induced by the mutation. A clear example of these mutated kinases is the product of the Philadelphia (Ph) chromosome, the BCR (breakpoint cluster region)–ABL (Abelson murine leukemia) fusion tyrosine kinase in CML. Accumulating data afterward demonstrated that tyrosine kinase signalling activation was induced by the mutation.
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JAK2 Mutation and Treatment of JAK2 Inhibitors

Generally, testing for the JAK2 V617F mutation includes allele-specific polymerase chain reaction (PCR) assay, pyrosequencing, restriction-enzyme digestion, and real-time PCR. The assays are sensitive to detect the presence of a heterozygous mutation in as few as 5 to 10% of cells and have low rates of false positivity. These properties make them useful for diagnostic purpose.

After the finding of V617F JAK mutant, many other mutations have been observed in JAK2V617F-negative MPNs, both in chronic phase (exon 12 mutations of JAK2, MPL, TET2, LNK, EZH2) and blast phase (NF1, IDH1, IDH2, ASXL1, CBL, Ikaros). Some of the mutations involve JAKSTAT signaling activation while others involve chromatin remodeling and leukemic transformation. One well-characterized mutation is the cytokine transmembrane receptor MPL (MPL W515). MPL W515 mutation is found in 3% patients of ET and about 10% cases with JAK2V617F-negative MPNs, both in chronic phase and blast phase (class II). Class I inhibitors primarily target JAK2 kinase (including JAK2V617F) whereas class II inhibitors were developed for non-MPD indications but still have therapeutic potential in MPD given their significant ‘off-target’ JAK2 kinase-inhibitory activity. For class I inhibitors, pharmacological strategy has been to refine an existing tyrosphostin (tyrosine phosphorylation inhibitor) scaffold based on available molecular structural data for JAK2 and JAK3 kinase domains, to design compounds that selectively bind to the JAK2 (relative to JAK3) kinase catalytic site at low nanomolar concentrations, as displayed in Figure 5. As a consequence, these compounds can potentially inhibit both wild-type (JAK2WT) and mutant JAK2V617F alleles. A cell-based screen was developed to identify allele-specific inhibitors of JAK2V617F-negative. These have the potential for a more optimal therapeutic window because the developed compound will only inhibit the disease allele. Vast majority of JAK2 inhibitors are small molecules that act by competing with ATP for the ATP-binding catalytic site in the kinase domain.

### Table 1. Frequency of the JAK V617F allele in myeloid disorders.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>Polycytemia vera</td>
<td>81-99%</td>
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<tr>
<td>Essential thrombocytosis</td>
<td>41-72%</td>
</tr>
<tr>
<td>Primary myelofibrosis</td>
<td>39-57%</td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia</td>
<td>3-9%</td>
</tr>
<tr>
<td>Myelodysplasia*</td>
<td>3-5%</td>
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<tr>
<td>Acute myeloid leukemia#</td>
<td>&lt;3%</td>
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*Most common in patients with refractory anemia with ringed sideroblasts and thrombocytosis, a clinically distinct subtype of myelodysplastic syndromes. Most common in patients with a previous history of polycythemia vera, essential thrombocytopenia and primary myelofibrosis.

Rational concept of JAK2 inhibitor treatment in MPD

Despite incomplete understanding of the molecular basis of MPNs, JAK2 remains an attractive target for drug development. Mutations with a gain of function of JAK2, MPL, and those with a loss of function of LNK and NF1 activate the JAKSTAT pathway leading to a final phenotype of MPN with alteration of immune response, inflammation, angiogenesis, proliferation and resistance to apoptosis. The character of resistance to apoptosis was supported by in vitro studies demonstrating that smallmolecule inhibitors of JAK2 inhibit the proliferation of cell lines carrying JAK2V617F. This inhibition is dose dependent and reduces the phosphorylation of JAK2 and STAT5 downstream signaling pathways resulting in induction of apoptosis. Even in patients without a confirmed JAK2 mutation, the detection of STAT activation indicates dysregulated JAK2 activity. Thus, regardless of the mutational status of JAK2, the malignant cells retain their responsiveness to JAK2-activating cytokines and/or growth factors and might benefit from JAK2 inhibition.

![Figure 5. Molecular structure of TG101209 and its selective inhibition of JAK2 kinase. (a) Chemical structure of TG101209 (molecular formula,C26H35N7O2S; molecular weight, 509.7; melting point 243°C). (b) Molecular model depicting docking of TG101209 in the JAK2 ATP pocket. The shaded surface illustrates hydrophilic (red) and hydrophobic (blue) portions of the protein. Key inhibitor–protein interactions, including the hydrogen bond with the hinge Leu932 residue, the hydrophobic contacts in the shallow angular pocket lined by residues Met929, the methylene groups of Lys882, and the initial portion of the DFG (AspPheGly) (AspPheGly shown) activation loop, as well as the hydrogen bond with the NH of the sulfonamide from TG101209 with Asn981 are shown. (c) TG101209 inhibitory activity (IC50) against select kinases in the in vitro kinase assay.](image-url)
Clinical evidence of JAK2 inhibitor in MPD
Clinical trials with small-molecule JAK2 inhibitors and drugs targeting other targets are displayed in Table 2.

Table 2. Current therapies for Ph-negative MPN patients. (modified from)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Target</th>
<th>Phase</th>
<th>Disease</th>
<th>Efficacy</th>
<th>JAK2V617F</th>
<th>Toxicity</th>
<th>Refs</th>
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<tbody>
<tr>
<td>JAK2 inhibitors</td>
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<tr>
<td>INCBO18424</td>
<td>JAK2</td>
<td>III</td>
<td>MF</td>
<td>&gt;50% reduction in splenomegaly and</td>
<td>JAK2V617F</td>
<td>Anemia</td>
<td></td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>JAK1</td>
<td></td>
<td>PV/ET</td>
<td>constitutional syndromes</td>
<td>load</td>
<td>Thrombocytopenia</td>
<td>12</td>
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<td>TGI10348 or SAR30250</td>
<td>JAK2,</td>
<td>II</td>
<td>MF</td>
<td>Reduction in splenomegaly</td>
<td>JAK2V617F</td>
<td>Anemia</td>
<td></td>
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<tr>
<td>FLT3</td>
<td></td>
<td></td>
<td></td>
<td>load</td>
<td>load</td>
<td>Thrombocytopenia Gastrointestinal</td>
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<tr>
<td>Cytarabine (Ara-C)</td>
<td>JAK2,</td>
<td>II</td>
<td>MF</td>
<td>Reduction in splenomegaly</td>
<td>JAK2V617F</td>
<td>Anemia</td>
<td></td>
</tr>
<tr>
<td>TK1052</td>
<td>JAK1,</td>
<td></td>
<td></td>
<td>load no</td>
<td>reduced</td>
<td>Thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>FLT3</td>
<td>TK2</td>
<td></td>
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<tr>
<td>CEP-701</td>
<td>JAK2,</td>
<td>II</td>
<td>MF</td>
<td>Reduction in splenomegaly</td>
<td>JAK2V617F</td>
<td>Gastrointestinal</td>
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<tr>
<td>lestaurtinib</td>
<td>FLT3</td>
<td></td>
<td>PV/ET</td>
<td>load</td>
<td>reduced</td>
<td>Anemia</td>
<td></td>
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<tr>
<td>Other targets</td>
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<td>LBH-580</td>
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<td>III</td>
<td>MF</td>
<td>Splenomegaly and anemia</td>
<td>unknown</td>
<td>Anemia</td>
<td></td>
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<tr>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Thrombocytopenia Gastrointestinal</td>
<td></td>
</tr>
<tr>
<td>RAJ001</td>
<td>FLT3</td>
<td>II</td>
<td>MF</td>
<td>Splenomegaly and anemia</td>
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<td>Minimal</td>
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<tr>
<td>Perifosine</td>
<td>IMID</td>
<td>III</td>
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<td>Anemia</td>
<td>unknown</td>
<td>Minimal</td>
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<tr>
<td>Pegylated Interferon</td>
<td>Biolog-</td>
<td>III</td>
<td>PV/ET</td>
<td>Thrombocytosis</td>
<td>unknown</td>
<td>Myelosuppression Depression</td>
<td>13</td>
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<tr>
<td>Alpha-2a</td>
<td>ical</td>
<td></td>
<td></td>
<td>symptoms</td>
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The best candidates for JAK2 inhibitor trial are intermediate-risk or high-risk PMF considering that the cases commonly present with significant disease-related morbidity such as cachexia or other constitutional symptoms, hepatosplenomegaly, extramedullary hematopoiesis, symptomatic anemia, and a poor prognosis with a median survival of 2-3 years. Currently, there is no effective therapy for these populations despite the introduction of immunomodulatory drugs. Few treatment options exist for patients with myelofibrosis (MF), and their survival is significantly shortened. The intention of conventional treatment is only palliative such as to prevent thromboembolism. In contrast, patients with ET or PV have a strikingly better life expectancy with median survival more than 20 years using conventional treatment in combination with phlebotomy and low-dose aspirin and hydroxyurea. In consequence, primary trial of JAK2 inhibitors in unselected ET or PV patients are not applicable until the safety and tolerability of these drugs have been confirmed in higher-risk MPN cases. INCBO18424, TGI10348, and CEP-701 are the drugs that are currently in the most advanced phases of testing. INCBO18424 and TGI10348 are the most promising JAK2 inhibitors with published results. The most intriguing data on JAK2 inhibitor came from a report published in 2010 by Verstovsek et al. on INCBO18424 (Ruxolitinib), a potent, selective, and orally bioavailable inhibitor of JAK1 and JAK2. Based on the fact that about half of patients with myelofibrosis carry JAK2V617F mutant, this trial was conducted as a phase II-II trial to compare patients with JAK2V617F- positive or JAK2V617F-negative PMF, post-ET MF and post-PV MF. This study was carried out in consideration that among other MPNs MF most associates with cytopenias, splenomegaly, poor quality of life and shortened survival. In a total of 153 patients with a median duration of more than 14.7 months, a dose-dependent suppression of phosphorylated signal transducer and STAT 3 was demonstrated in either patients with wild-type JAK2 or with JAK2V617F mutation. With a 15-mg twice-daily starting dose, followed by individualized dose titration, 17 of 33 patients (52%) had a rapid objective response (≥ 50% reduction of splenomegaly) which persisted for 12 months or more. This therapy was associated with grade 3 or grade 4 adverse events (mainly myelosuppression) in less than 10% of patients. In addition, clinical improvement was associated with a marked reduction of levels of circulating inflammatory cytokines that are commonly elevated in MF. Later on, an MF-specific instrument called Myelofibrosis Symptom Assessment Form was proposed to help characterize the symptomatic improvements observed in trials on MF patients. The reduced constitutional symptoms have been attributed to dual activity of Ruxolitinib against JAK1 and JAK2. JAK1 inhibitory activity of RuxolitinimbTY3 contributed to the suppression of secretion from several cytokines (e.g., IL-6), which is thought to be responsible for the induction of constitutional symptoms. TGI10348 is an oral JAK2 inhibitor that has been tested in a phase I trial on 59 patients with PMF or post-PV, post-ET MF. This study recruited intermediate and high-risk patients being unresponsive to standard treatments. The subjects presented with thrombocytopenia (platelet less than 50 x10^9/L) and neutropenia (actual neutrophil count less than 1,000 x10^9/L). The maximum tolerated dose was 680 mg/day and dose-limiting toxicity was a reversible and asymptomatic increase in the serum amylase level. Dose chosen for a phase II/III trial was 400 mg or 500 mg daily. Based on (International Working Group)IWG-MRT criteria of response, 59% of patients achieved reduction of spleen size by palpation at 6 months. The majority of patients with constitutional symptoms, fatigue and pruritus had a durable improvement. However, these decreased parameters were not associated with a measurable effect on cytokines. Between different doses, leukocytosis and thrombocytosis were normalized at 12 months in 57% and 90% of patients. There were no differences in term of response rate according to JAK2V617F mutational status. Of all patients, 39 cases with more than 20% mutant JAK2V617F/total JAK2 ratio (allelic burden) at baseline showed decreased mutation load more than 50% at 12 months. The toxicities included grade 3-4 hematologic events such as anemia (in 35% of 37 patients who were not RBC.
transfusion dependent at baseline, thrombocytopenia (24%) and neutropenia (10%). The main non-hematologic adverse events included all grades nausea (69%), diarrhea (64%) and vomiting (58%). These reactions were all self-limiting and controlled by symptomatic treatments. Asymptomatic increase of lipase, AST, ALT and creatinine have been observed in about 25% patients.

CEP-701 (Lestaartimib) is a non-selective small-molecule inhibitor of TRK that was initially developed for treatment of prostate cancer. In vitro, it inhibited JAK2 kinase, the proliferation of progenitor cells and JAK2STAT5 signaling from patients with MPNs. A phase II study investigated the efficacy of CEP-701 in PMF patients. Santos et al. reported CEP-701 treatment in 22 JAK2V617F-positive MPF patients (80 mg orally twice daily). Most patients (90%) were previously treated and the median number of previous therapies was 3 (range from 0-6). Splenomegaly was present in 90% of patients with 19 cm of median size from left costal margin (range from 0-30 cm). Median allele burden was 53.5%. Six patients (27%) responded by IWG criteria (clinical response in all cases). Clinical responses included reduction in spleen size only (n = 3), transfusion independency (n = 2), and reduction in spleen size with improvement in neutrophil counts and platelets (n = 1). Median time to response was 3 months and duration of response was 214 months. No improvement was seen in bone marrow fibrosis or JAK2V617F allele burden. Phosphorylated STAT3 levels decreased from baseline in responders during therapy. Eight patients (36%) experienced grade 3 or 4 toxicity and 6 (27%) required dose reduction. Main side effects were myelosuppression (grade 3 or 4 anemia, 18%; and thrombocytopenia, 18%) and gastrointestinal disturbances (diarrhea, any grade, 68%; grade 3 or 4, 9%; nausea, grade 1 or 2 only, 50%; vomiting, grade 1 or 2 only, 27%). Overall, CEP-701 resulted in modest efficacy and a relatively well tolerated toxicity in MP patients.

Based on the underlying genetic mechanism, patients with MPNs showed a various response to different treatment. As much as 40-50% of the patients with PMF and ET who carried JAK2V617F mutation had decreases in proportion of JAK2-mutated DNA after treatment. About 20% of the PMF and ET patients who carried MPL mutations had no decreases in proportion of MPL-mutated DNA when treated with JAK2 inhibitors.

As new alleles are identified, either alone or in conjunction with JAK2 mutations, additional drugs may evolve that target these alleles. There is also space for development of drugs that have been empirically used. Recently, other drugs targeting alternative pathways which are critical for MPN development were reported on trials. These include inhibitors of chromatin remodeling proteins such as histone deacetylase inhibitors and HSP90 inhibitors, or the drugs acting through remodeling chromatin with a key role in epigenetics (givinostat, panobinostat, vorinostat); pegylated interferon alpha-2a, mammalian target of rapamycin inhibitor/mTOR (everolimus), the MAPK (erlotinib) and the NF-Kb (bortezomib) pathways. Combination of JAK2 inhibitors with other regimens which show synergy and other biological properties than JAK2 inhibitors holds promise as effective treatment in these disorders.

Expert opinions and further considerations

The genetic, biochemical and functional studies have described important insight into the pathogenesis of PV, ET and PMF as Ph-negative MPNs. The discovery of the JAK2V617F mutation has also strengthened the association between the three diseases. However, there are still essential questions regarding the molecular basis of disorders. The role of a single disease allele in three related but clinically distinct phenotypes is not well understood. Thus, future studies will undoubtedly discover additional mutations that contribute to the pathogenesis of these MPNs. The identification of the JAK2V617F mutation has provided a pivotal basis for the development of JAK2-targeted therapies. Recently a growing number of trials have tested JAK2 inhibitor aimed at determining the safety and activity of these agents. Ruxolitinib (INCBO18426) has proven safe and has been shown to be effective at reducing spleen size and improving clinical symptoms in patients with MF. Ruxolitinib has also proven very effective in patients with PV and ET. Similar results in PMF have been obtained with TG101348 and cyt387. Furthermore, improvements in cytopenia and bone marrow fibrosis, as well as complete molecular responses in JAK2V617F-positive patients still need to be further explored. The role of JAK2V617F mutation in ET and PMF as the driving force in disease mechanism is somewhat unclear. Nevertheless, the dramatic improvement in quality of life and splenomegaly achieved by JAK2 inhibitors in patients with MPN supports the notion that these agents may become the new standard of therapy in PMF. As the understanding of the mechanism of transformation by JAK2V617F is incomplete, the data showing that JAK2V617F-negative AML occurs frequently in patients with a JAK2V617F-positive MPN, may raise the possibility that JAK2 inhibitor therapy might increase the risk of leukemic transformation. Longer investigation will define whether treatment with JAK2 inhibitor can prolong survival, reduce the risk of thrombotic events and transformation to AML. These findings may therefore change the natural history of MPN. In population of patients with PV management using conventional therapies such as aspirin, aspirin and hydroxyurea has a reasonable outcome at relatively modest cost. A cost-benefit analysis of potentially expensive long-term targeted therapy is thus highly needed.

REFERENCES


