Clinical Applications of Molecular Haematology: 
**JAK2 in Myeloproliferative Disorders**

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**Abstract**
Molecular markers are helpful in diagnosis, prognosis and management of haematological malignancies. Recently, a single point mutation in the Janus Kinase 2 (JAK2) gene in the Philadelphia-negative myeloproliferative disorders, including polycythemia vera (over 95%), essential thrombocytemia (50%) and primary myelofibrosis (50%) was identified by several groups. This mutation is now considered to have a fundamental role in the pathogenesis of these disorders. A PCR-based test from peripheral blood has become available in India to detect this mutation. Present article discusses the basic aspects of this mutation and its value in diagnosing, prognosticating and treating patients of suspected chronic myeloproliferative disorders.

**INTRODUCTION**

Dr. William Dameshek, in 1951, coined the term myeloproliferative disorders (MPDs) to respect the overlapping clinical and laboratory features of polycythemia vera (PV), essential thrombocytemia (ET), myelofibrosis with myeloid metaplasia (MMM) and chronic myeloid leukemia (CML). What Dr. William Damashek had thought of 55 years earlier, has now been proven to be more prescient than what haemato-oncologists had previously realized.

**JAK2:**

Blood cell production is regulated by a number of protein growth factors and cytokines. They are responsible for cell survival, proliferation and differentiation. These molecules bind to cell surface receptors. The receptors are intimately associated with tyrosine kinases of the Janus kinase (JAK) family. There are four mammalian JAKs: JAK1, JAK2, JAK3 and TYK2. The JAK-STAT signaling pathway has great significance in pathophysiology of MPDs. The JAK family of tyrosine kinases transmits signals from cytokine receptors and activate intracellular signaling pathways, most notably by engaging the STAT family of signal transduction proteins. Activation of this signaling pathways results in the development of an MPD. Figs. 1 and 2 delineates the overall scheme of JAK, signal transducers and activators of transcription and suppressor of cytokine signaling family members showing important domains. They also show JAK2V617F mutation and the key regulatory phosphorylation site (Y1007).

**JAK2 and myeloproliferative disorders:**

The identification of JAK2V617F mutation in MPDs, in 2005, was a major breakthrough in our understanding of the pathogenesis of MPDs, especially PV, ET and MMM. It can be compared with identification of bcr-abl fusion gene in the pathogenesis of chronic myeloid leukemia (CML) way back in 1985. Five groups, independently, within a year i.e. 2005, using different techniques, identified and reported the presence of a single mutation in the JAK2 tyrosine kinase (JAK2V617F) in different non-CML MPDs with almost remarkable consistency with respect to the frequency.

Table 1 enlists the frequency of JAK2V617F mutation in PV, ET and MMM from four of these studies. The investigators noted absence of JAK2V617F mutation in germline DNA from MPD patients, demonstrating that the mutation is an acquired somatic mutation in a haematopoietic progenitor.

In JAK2V617F mutation, valine at Codon 617 is substituted by phenylalanine. This results in a loss of autoinhibition of JAK2 activity. JAK2V617F mutation has the ability to transmit signals from the erythropoietin (EPO) receptor, the thrombopoietin (TPO) receptor and the granulocyte-colony stimulating factor (G-CSF) receptor in haematopoietic cells, more efficiently.

<table>
<thead>
<tr>
<th>Study</th>
<th>PV (%)</th>
<th>ET (%)</th>
<th>MMM (%)</th>
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<tbody>
<tr>
<td>James et al</td>
<td>89</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Kralovic et al</td>
<td>65</td>
<td>23</td>
<td>57</td>
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<tr>
<td>Baxter et al</td>
<td>97</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>Levine et al</td>
<td>74</td>
<td>32</td>
<td>35</td>
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Note: Higher frequency of JAK2V617F mutation in the study by Baxter et al is due to the use of a more sensitive technique i.e. allele-specific PCR methodology.
Laboratory detection of JAK2 mutation and its significance:

Sensitive and specific laboratory techniques have developed to detect JAK2\(^{V617F}\) mutation using peripheral blood neutrophils. These include allele-specific PCR, pyrosequencing and quantitative real-time PCR.\(^3\) A positive test strongly favours MPD as it is usually negative in normal individuals, secondary erythrocytosis, reactive thrombocytosis, leukaemoid reaction and secondary myelofibrosis.\(^3\) The test is also negative in normal B-lymphocytes, normal T-lymphocytes, lymphomas and sarcomas.\(^3\) It is usually negative in chronic myeloid leukaemia, acute myeloid leukaemia (unless those evolving from MPD) and myelodysplastic syndrome except for those associated with features of MPD or 5q- where thrombocytosis is common.\(^3\) Besides, the test may also be useful as a sensitive marker detecting MPDs as the underlying aetiology of splanchnic venous thrombosis affecting splenic, portal and hepatic veins.\(^3\)

How does a single mutation result into three distinct blood disorders, remains an enigma. Similarly, although almost all patients with PV (95%) and nearly half of patients with ET and MMM are JAK2\(^{V617F}\) mutation - positive, why do the remaining patients with PV, ET and MMM have no detectable JAK2\(^{V617F}\) mutation, remains unclear? How does one explain this?

There are at least three possible explanations

1. The stringency of the criteria used to diagnose PV, ET and MMM
2. The sensitivity of the method used to detect JAK2\(^{V617F}\) mutation
3. The source of DNA. The mutational analysis is typically performed on DNA from neutrophils. It is possible that the JAK2\(^{V617F}\) mutation is confined to the red cells or platelet compartment in some patients.

Also, it is possible that some patients are truly JAK2\(^{V617F}\) mutation - negative. There may also be alternative somatic mutation(s).

Clinical and laboratory features of JAK2 Positive MPDs:

Clinical features of JAK2\(^{V617F}\) mutation - positive MPD are being delineated. JAK2\(^{V617F}\) mutation positive ET patients differ from those who are negative as they have higher haemoglobin (Hb), lower platelet counts, lower serum erythropoietin (EPO) and lower serum ferritin. This suggests that ET patients with JAK2\(^{V617F}\) mutation are closer to patients with PV. They also exhibit higher risk of venous thrombosis. It has also been shown that in JAK2\(^{V617F}\) mutation-positive ET patients, treatment with hydroxycarbamide is superior to anagrelide in decreasing the risk of venous thrombosis. As against this, anagrelide is superior for JAK2\(^{V617F}\) mutation - negative ET patients. It has been suggested that patients with JAK2\(^{V617F}\) mutation-positive ET are a forme fruste of PV with the level of erythrocytosis influenced by genetic or acquired modifiers.\(^3\)

In patients with MMM, JAK2\(^{V617F}\) mutation has been linked with higher white cell count and an older age at presentation as well as with pruritus and thrombosis. The JAK2\(^{V617F}\) mutation positive patients with MMM have reduced red cell transfusion requirement and relatively poor survival.

Patients with PV and homozygous JAK2\(^{V617F}\), have higher Hb at diagnosis and increased rate of fibrotic change, but not thrombosis or bleeding. Homozygous mutations are uncommon in ET compared with PV.

Certain workers have investigated patients with familial MPD.\(^10\) In majority of them, acquired mutations in JAK2 were identified. These results suggest that there are inherited “pre-JAK2” alleles that predisposed to
the development of JAK2V617F mutation-positive MPDs. These inherited mutations remain to be identified. In paediatric age group, MPDs can present as sporadic or familial diseases. JAK2V617F mutation is less frequent in paediatric PV than in adult PV. Children with familial ET (as against sporadic ET) do not have JAK2V617F mutation.

JAK2 antagonists in treatment of MPDs:

The discovery of JAK2V617F has great diagnostic and clinical significance that will further heighten in years to come. It is clear that in view of very high frequency of JAK2V617F mutation in PV, incorporating a mutation screen early into the algorithm for investigating a case of erythrocytosis could help to streamline diagnosis of PV vs secondary erythrocytosis (Fig. 3). This may reduce the need for further investigations such as red cell mass and bone marrow biopsy. Similarly, in patients with thrombocytosis, the use of JAK2V617F mutation analysis may assist in identifying patients with a stem cell disorder. With the advent of these molecular markers for MPDs including CML, a new classification of MPDs is on card as depicted in Fig. 4.

Development of imatinib mesylate (IM) by Dr. Brian Druker for the treatment of CML had its basis in the identification of bcr-abl fusion tyrosine kinase which had occurred almost two decades earlier. There is optimism that the discovery of JAK2 mutation will lead to development of similar specific pharmacologic inhibitors of JAK2 with the potential to transform the treatment of PV, ET and MMM.

However, there are few questions that need careful evaluation. First, whether it is possible to obtain a drug having preferential activity against mutant rather than wild type JAK2 (WT JAK2) and which does not produce significant haematological toxicity. Secondly, as the management of patients with PV using conventional therapies i.e. venesection, aspirin and hydroxyurea, has a reasonable outcome at relatively modest cost, a cost-benefit analysis of potentially expensive long-term targeted therapy is needed. Thirdly, in ET and MMM, the role of JAK2V617F mutation as the driving force in disease pathophysiology is far from clear and hence the impact of JAK2-directed therapy remains uncertain.

Erlotinib effectively inhibits JAK2V617F activity and polycythaemia vera cell growth. It has little effect on normal cells. It is possible that Erlotinib may be used for treatment of JAK2V617F - positive PV and other MPDs.

Acknowledgement

Based on presentations made at the "XIIIth National CME in Haematology" held at Bombay Hospital, Mumbai in January 2007.

REFERENCES


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**Announcement**

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