

# Immunotherapy and polycythemia vera

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## Abstract

Polycythemia vera (PV) is a chronic myeloproliferative disorder characterized by abnormal growth of erythroid precursors in the bone marrow. Almost all patients with PV, around 97%, have a mutation in Janus kinase 2 (JAK2). It is through the activation of JAK/Signal Transducers and Activators of Transcription (STAT) protein signaling pathway that the JAK2 mutation is thought to induce cellular proliferation, growth, hematopoiesis and immune response in PV patients. A summary of the current role of immunotherapy in the treatment of PV is provided. There are new JAK kinase inhibitors that are currently being evaluated and are at various stages of clinical trials and development, as well as the previously studied cytokines therapies. At the present time, ruxolitinib and interferon- $\alpha$  (IFN- $\alpha$ ) are the only United States Food and Drug Administration (FDA) approved drugs for the management of advanced PV. JAK kinase inhibitors are better tolerated and less problematic than the interferons. Still, allogeneic stem cell transplantation is the only potentially curable method for end-stage PV. Additional genetic mutations have been implicated in PV pathogenesis. In this perspective, targeting different pathways might be required. Further investigations are needed to evaluate the promising role of immunotherapy in PV whether alone or in combination with other modalities.

**Abbreviations:** AE: Adverse Events; Bcl-x: B-cell lymphoma-extra; CMML: Chronic Myelomonocytic Leukemia; CYP: Cytochrome P; ET: Essential Thrombocythemia; FDA: Food and Drug Administration; HSC: Hematopoietic Stem Cells; IFN- $\alpha$ : Interferon alfa; JAK2: Janus Kinase 2; JAK/STAT: Janus Kinase /Signal Transducers and Activators of Transcription; MPDs: Myeloproliferative Disorders; MU: Million Units; NF-E: Nuclear Factor Erythroid-Derived 2; PEG: Polyethylene Glycol; PD/PK: Pharmacodynamics/Pharmacokinetics; PMF: Primary Myelofibrosis; PV: Polycythemia Vera; PRV-1: Polycythemia Rubra Vera 1; Tpo-R: Thrombopoietin Receptor; Wt: Wild Type

## Introduction/Epidemiology

Myeloproliferative disorders (MPDs) are classified according to the most affected type of blood cells. There are four main types of MPDs, which include Polycythemia Vera (PV), Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF), and Chronic Myelomonocytic Leukemia (CMML).

PV is a myeloproliferative disorder, which presents as an abnormal increase in the number of red blood cells [1]. It is the most common form of the MPDs. Its incidence in the US is estimated to be 1.9/100,000, with an increase associated with ageing. The mean age at diagnosis of PV is approximately 60 years old. However, in 2010 the age adjusted prevalence of PV was 57.15/100,000 [2]. PV is very rare in children.

## Etiology/predisposing risk factors

The cause of chronic MPDs remains unknown. A mutation in a specific protein, Janus kinase 2 (JAK2) is found in a large number of people with MPDs. It is detected in about 95% of those with PV, in approximately 50–70% with ET, and 40–50% with PMF [3]. There are several risk factors, which increases the risk of chronic MPDs. They include the following:

## Age and sex

Although one third of the cases diagnosed are in those below the

age of 50, PV is most commonly diagnosed at 60 years of age and above [4]. Similarly, the incidence of MPDs and specifically CMML increases with age [5,6]. The median age at diagnosis of CMML patients ranges between 65 and 75 years [7]. PV, as well as all MPDs in general, are more prevalent in men [5].

## Cancer treatment

Prior treatment with chemotherapy appear to increase the risk of CMML [8]. However, the risk of CMML after cancer chemotherapy is not as high as that of other hematological malignancies, such as myelodysplastic syndromes and acute myeloid leukemia [9].

## Exposure to carcinogenic agents

Petrochemicals such as benzene and toluene, and ionizing radiation increase the risk of PV and PMF [10,11]

## Pathophysiology/molecular basis

A mutation in a specific gene, Janus kinase 2 (JAK2) is found in a large percentage of people with MPDs. Figure 1 describes a working model for genetic events and other mechanisms, possibly involved in the pathophysiology of MPDs [12].

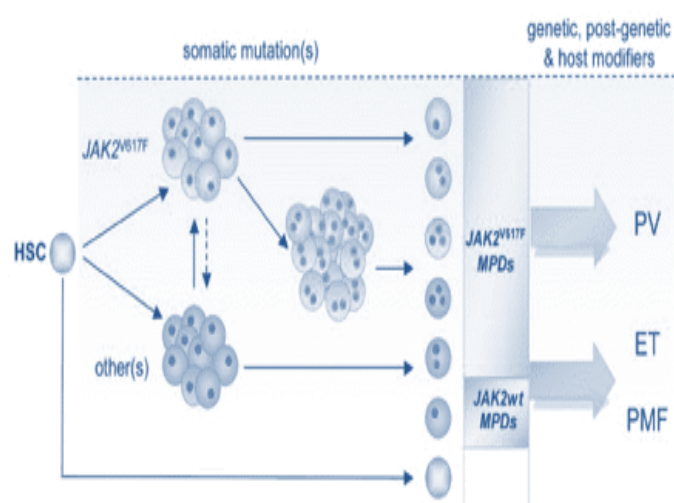
## Hematopoietic growth factor responses mediated by the JAK/STAT Pathway

The JAK/STAT pathway [13] plays a vital role in the initiation of

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**Figure 1:** A model of genetic events and other mechanisms possibly involved in the pathophysiology of MPDs HSC: Hematopoietic Stem Cells; MPDs: Myeloproliferative Disorders; PV: Polycythemia Vera; ET: Essential Thrombocythemia; Wt: Wild Type; PMF: Primary Myelofibrosis.

signal transduction through hematopoietic growth factor receptors. JAK/STAT is also a target for identifying the molecular abnormalities in PV. Furthermore, in erythropoietin-independent differentiation of erythroid progenitors in PV, where constitutive activation of STAT3 has been reported, STAT3 was found to be repressed by inhibitors of JAK2 [14,15]. Recently, several groups have identified a consistent, single somatic activating mutation in the *JAK2* gene in the majority of patients with PV [16].

### JAK2 mutation in the PV

Several studies showed that the majority of patients (65%-97%) with PV have the *JAK2*-V617F mutation [17,18]. A report by Kralovics *et al.* [19] showed that patients with *JAK2*-V617F had a significantly longer duration of disease, more treatment with cyto-reducing agents and higher rates of complications (*i.e.*, myelofibrosis, thrombosis and bleeding) than those with wild-type *JAK2*. Other biologic and epigenetic markers present in PV patients are deregulated expression of Bcl-x (B-cell lymphoma-extra, an inhibitor of apoptosis) [20], overexpression of the *PRV-1* (polycythemia rubra vera 1) and transcription factor *NF-E2* (nuclear factor erythroid-derived 2) genes [21,22], and impaired expression of Tpo-R (thrombopoietin receptor) [23]. No mutations have yet been detected in these genes and recent evidence indicates that altered expression of these markers is due to activation of the JAK/STAT pathway through the *JAK2*-V617F mutation [24]. Therefore, abnormal expression of these molecules appears to be a secondary consequence of the primary *JAK2* mutation [25].

### Molecular Re-classification of the MPDs

From the well-known phenotypic overlap and transitions that occur during the natural history of the classic MPDs, it is also possible that patients with clinically diagnosed ET or myelofibrosis, who have the *JAK2*-V617F mutation, actually represent variants of PV [25,26]. This possibility is supported by the finding that up to 30% of patients with PV, but very few patients with ET, appear to be homozygous for *JAK2*-V617F [25]. Therefore, mutation-positive ET patients may, in fact, be individuals with “inapparent PV”. These patients may have variable degrees of plasma volume expansion which might mask an absolute increase in red cell mass, unless these are directly measured

[27], the distinction is not always perceptible. They may also represent early or late (spent) phases of PV.

## Immunotherapy for PV

### Kinase inhibitors

**Ruxolitinib:** An orally bioavailable JAK inhibitor with potential antineoplastic and immunomodulating activities approved by FDA in the management of PV. Ruxolitinib specifically binds to and inhibits protein tyrosine kinases, JAK1 and JAK2, which may lead to a reduction in inflammation and an inhibition of cellular proliferation. The JAK/STAT pathway plays a key role in the signaling of many cytokines and growth factors and is involved in cellular proliferation, growth, hematopoiesis and the immune response; JAK kinases may be up-regulated in inflammatory diseases, MPDs and various malignancies.

Ruxolitinib is FDA approved for the treatment of patients with PV, who have had an inadequate response to or are intolerant to therapy with hydroxyurea. Ruxolitinib has also been indicated for the treatment of intermediate or high-risk myelofibrosis, including primary myelofibrosis, post-PV myelofibrosis and post-ET myelofibrosis and PV in patients who have had an inadequate response to or are intolerant to hydroxyurea.

Orally administered ruxolitinib is rapidly absorbed with  $C_{max}$  achieved within 1-2 hours post-administration. The mean volume of distribution at steady-state is 75 L with an inter-subject variability of 23%. Ruxolitinib is metabolized by cytochrome P3A4 (CYP3A4) with a mean elimination half-life of approximately 3 hours. Adverse events may include thrombocytopenia, anemia and neutropenia, risk of infections, and non-melanoma skin cancer. However, the most common hematologic adverse reactions are thrombocytopenia and anemia with the most common non-hematologic adverse reactions reported being bruising, dizziness, and headache [28].

**Momelotinib:** Momelotinib is an orally bioavailable small-molecule inhibitor of JAK1/2 with potential antineoplastic activity, which is being investigated in a phase II clinical trial (Table 1). Momelotinib competes with JAK1/2 for ATP binding, which may result in the inhibition of JAK1/2 activation, and therefore, inhibition of the JAK/STAT signaling pathway, thus promoting induction of apoptosis and a reduction of tumor cell proliferation in JAK1/2-expressing tumor cells.

### Cytokine therapy

**Interferon-alfa (IFN-α):** IFN-α is an analogue of consensus interferon containing an additional methionyl-amino-acid residue. Consensus interferon (also known as interferon alfacon-1; rCon-IFN, and C1FN) is a genetically engineered synthetic interferon created from the most common amino acid sequences found in the naturally occurring alfa interferons. Alfa interferons bind to specific cell-surface receptors, resulting in the transcription and translation of genes whose protein products have antiviral, antiproliferative, anticancer, and immune-modulating effects.

IFN-α suppresses the proliferation of hematopoietic progenitors and has a direct inhibiting effect on bone marrow fibroblast progenitor cells. Additionally, IFN-α antagonizes the action of platelet-derived growth factor, transforming growth factor-β and other cytokines that may be involved in the development of myelofibrosis. IFN-α should be administered at the dose of 3 million units (MU) daily, until a hematocrit response is reached (hematocrit < 0.45 [45%]); then, maintenance therapy has to be adjusted to the lowest weekly doses

**Table 1.** Non-FDA Approved kinase inhibitors [29].

Drug	Clinical trial identifier no.	Phase	Study Design	Target
Momelotinib	NCT01998828	Phase II	Randomized, Safety/Efficacy study, open label	JAK1/2

**Table 2.** Non-FDA Approved IFN- $\alpha$  [32,33].

Drug	Clinical trial identifier no.	Phase	Study Design	Target
PEGASYS	NCT01259856	Phase III	Randomized, Safety/Efficacy study, open label	Cell surface receptors of the malignant clone in PV
PEGASYS	NCT01259817	Phase II	Non Randomized, single arm, open label, Safety/Efficacy Study	Cell surface receptors of the malignant clone in PV

that control the hematocrit at that response level. During the first month of therapy a complete hemogram must be recorded weekly; for the second month the test is required, every 2 weeks, then monthly, and, in steady-state in responding patients, every 3-4 months. IFN- $\alpha$  is contraindicated in patients with thyroid and/or mental disorders. The most common adverse effects associated with IFN- $\alpha$  are weakness, myalgia, weight and hair loss, severe depression, and gastrointestinal and cardiovascular symptoms [30,31].

**PEGASYS:** PEGASYS is a covalent conjugate of recombinant IFN- $\alpha$ , subtype 2a and polyethylene glycol (PEG), used as an antiviral and antineoplastic agent and presently being tested in a phase II clinical trial (Table 2). The biological activity of this agent is derived from its IFN- $\alpha$ -2a protein moiety. IFN- $\alpha$  binds to specific cell-surface receptors leading to the transcription and translation of genes whose protein products mediate antiviral, antiproliferative, anticancer and immune-modulating effects. The PEG moiety lowers the clearance of IFN- $\alpha$ -2a, thereby extending the duration of its therapeutic effects, but may also reduce interferon-mediated stimulation of an immune response.

**Stem cell transplantation:** Currently, allogeneic stem cell transplantation is the only potentially curative treatment for advanced PV. Analysis of 250 consecutive patients was performed with an initial diagnosis of PV ( $n=120$ ) who underwent transplantation due to progression of myelofibrosis ( $n=193$ ) or acute myeloid leukemia ( $n=57$ ) and who were reported to the European Group for Blood and Marrow Transplantation registry between 1994 and 2010. The median age was 56 years (range, 22–75), and the interval between diagnosis and transplantation was 10 years or more in 52 % of the cases. With a median follow-up from transplantation of 13 months, the 3-year overall survival rate and relapse incidence were 55% and 32%, respectively. In a univariate analysis, the main parameters that negatively affected post-transplantation outcomes were age ( $>55$  years), a diagnosis of acute myeloid leukemia at transplant and obtaining a suitable transplant from an unrelated donor. The overall 3-year cumulative incidence of non-relapse mortality was 28%, but was significantly higher in older patients than in younger ones ( $>55$  years, 35% versus 20%,  $P=0.032$ ), in those transplanted from an unrelated donor rather than a related donor (34% versus 18%,  $P=0.034$ ) and in patients with a diagnosis of acute myeloid leukemia compared to myelofibrosis (29% versus 27%,  $P=0.045$ ). This large retrospective study confirmed that transplantation is potentially curative for patients with end-stage PV progressing to myelofibrosis or acute myeloid leukemia.

I looked up the topic and I could not find planned clinical trials involving the use of vaccination the management of PV or MPDs (except CML) [34].

## Conclusions and future perspectives

Our success in treating hematological malignancies is increasing and advancing day by day with improved knowledge of how the

immune system becomes dysfunctional in these blood disorders. The identification of the JAK2-V617F mutation in patients diagnosed and progressing to chronic MPDs has stimulated a great deal of effort in screening for MPDs and for developing specific JAK1/2 and other inhibitors for clinical therapy. It is certain that the next few years will bring further developments in this fast-evolving field. Researchers are still challenged to explore innate and adaptive immune systems and immunotherapy has become a promising development in the past few years in the treatment of various cancers. These recent advances have increased our understanding of the tumor microenvironment, and of various immunotherapeutic modalities or combination therapies such as chemotherapy with immunotherapy although the effects of such modalities in combination with immunotherapy in cancer patients are still in the exploratory phase. In summary, the complete perspective of immunotherapy treatment has not been realized and/or utilized and in that respect proper preclinical and clinical designs are the important pillars for understanding the future of immunotherapy in treating cancer patients and individuals with hematological disorders.

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