Polycythaemia vera: molecular genetics, diagnostics and therapeutics

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Abstract
Polycythaemia vera is one of several classical myeloproliferative neoplasms that may occur in a juvenile onset or late-onset adult forms. It is linked to specific genetic mutations that cause a deleterious elevation in the patient’s red cell mass. The discourse on genetics includes an exposé on the molecular biology of the disease and how a shared JAK2 V617F mutation can co-exist among three distinct neoplasms. Concepts of genetics and immunology help define the origin and behaviour of the disease: the tracking of allele burdens of mutations (genetic dosage), the timing or order of acquired mutations, the import of bystander mutations and the onco-inflammatory response; all theories are invoked to explain the progression of disease severity and potential transformational leukaemia. The World Health Organization’s diagnostic criteria are accessed to focus on the subtleties of the Hb laboratories and sifting through the challenging listing of differential diagnoses that mimic PV, and our report includes an overview of manual and automated phlebotomy (erythrocytapheresis) procedures, enumerating their clinical indications, significance of temporary phlebotomy resistance and optimizing safety/efficacy, quality and cost. Stratification of low and high-risk disease distinguishes when to commence chemo-cytoreductive therapy in the high-risk patient to prevent thrombotic complications. Drug resistance is circumvented by artfully switching drugs or using novel drug designs.

Key words: paediatric/juvenile/adult polycythemia vera, myeloproliferative neoplasms, JAK2 V617F mutations, JAK inhibitors, tyrosine kinase inhibitors.

Introduction
Polycythaemia vera (herein referred to as PV) is a myeloid neoplastic multi-clonal stem cell disorder. Cytogenetically, PV is a myeloproliferative neoplasm (herein referred to as MPN), classically categorized as BCR-ABL1 (Philadelphia chromosome) negative. Essential thrombocythaemia (herein referred to as ET) and myelofibrosis (MF) are categorized as sister diseases, being also BCR-ABL1 (Philadelphia chromosome) negative.

The hallmark of PV is the patient who frequents the clinic cyclically for phlebotomy treatments. This is because of their high red cell mass RCM which can be >25% above the predicted value caused by excess red cell production [1]. Because such patients are at heightened risk for thrombotic complications, it becomes necessary to remove the excess red cells periodically from their circulation.

With a male predominance, polycythaemia vera can start in juveniles (age < 20) or adults (age 60–65), although more commonly in the latter [2,3]. In later life after the diagnosis of PV, some patients transform to a diagnosis of MF and have an increase in grade 2 bone marrow fibrosis. They are at risk of adult onset acute myeloid leukaemia (herein referred to as AML). This malignancy is known to occur in 25–50% of adults with
significant bone marrow fibrosis and has a devastatingly poor prognosis for some patients [4]. Risk factors for leukaemic blast phase crises in PV are myelofibrosis, abnormal karyotype, TP53 and TET2 and DNMT3A genetic mutations [5–7]. This blast crises occurs in 10% of the patients within 10 years of their diagnosis of PV [6].

Molecular genetics of polycythaemia vera in a mutated Janus kinase 2 gene

The original 2005 discovery of the mutated Janus kinase 2 gene is on human chromosome 9. It effects the abnormal translation of JAK2 protein kinase associated with MPNs including PV [8,9]. In the schema of the Janus family of non-receptor protein kinases, there are four of them enumerated Janus kinase 1 (JAK1), Janus kinase 2 (JAK2), Janus kinase 3 (JAK3) and tyrosine kinase 2 (TYK2) [10].

A principal focus is the derangement of the JAK2 gene, cytogenetic localization 9p24.1, causing an activated/dysregulated JAK2 protein kinase and myelosis [11].

When DNA mutations occur in specific peptide coding exons 14 and 12 of the JAK2 gene, errors are made in protein translation and errant amino acid sequences inserted into the JAK2 protein kinase. The first discovered mutated protein JAK2 kinase V617F is related to a single DNA point mutation G1849T of exon 14 from the transversion of guanine to thymine. This leads to the replacement of the amino acid valine by the larger phenylalanine at codon 617 is predicted by molecular modelling to destabilize the pseudokinase domain JH2 [15]. Structural destabilization of the pseudokinase domain is thought to favour gain-up (constitutive activation) of the adjacent JH1 catalytic domain [4,16]. The gain in function is associated with increased disease activity as reported by several investigators manifested by pruritus, erythrocytosis, required advanced treatment intensity by cytoreductive therapy, and the propensity to fibrosis [8,17,18].

An interesting observation is that some cases of PV are negative for JAK2 mutations raising the possibility of other mutations elsewhere in association with the disorder. There are reported true cases of PV with TET2-first mutations, cytogenetic localization 4q 24, but negative for the JAK2 mutations [19].

Developmental theories of the myeloproliferative neoplasms

As separate disorders, an interesting question is the relationships between ET, PV and MF, given many MPN patients having a shared JAK2 V617F mutation. One theory is the report of mutant allele burdens that cumulate in the same patient with disease transformation over time, as highlighted below [20]. The quantitative allele burden is defined as the ratio of the mutated allele over the wild type (non-mutated). In one young woman in her early 20s, ET was diagnosed (disease time-line 0–3–5 years, Plt: 800 × 10^9 l^-1), transformed to PV (disease time-line 3–5–10 years, Hb: 18.3 g/dl, JAK2 V617F allele burden: 24-8%) bHb transformed to MF (disease time-line 10–12 years, WBC: 19 × 10^9 l^-1, JAK2 V617F allele burden 63-3%). The escalated gene dosage of mutated JAK2 correlated historically with a staged progression from thrombocytosis to erythrocytosis to granulocytosis.

Enigmatically, some ET cases bypass transformation to PV. Some of these ET patients whether mutated or not evolve to fibrosis and AML directly and the thrombotic risk escalates when age ≥60 with JAK2 mutation [21]. A plausible explanation why disease sometimes jumps steps is that the specific type and timing or order of somatic mutations in the population define the neoplasm [22]. In
A smaller cohort study of 24 total patients with chronic phase MPNs, those with acquired JAK2-first mutations were compared to 12 patients with acquired TET2-first mutations (22% associated frequency with PV) [17,22,23]. Clonal studies of the mutations were performed in cultured burst forming erythroid units BFU-E. Those with JAK2-first had a predilection to a diagnosis of PV, 7 of the 12 patients with the balance of 5 patients ascribed to ET and MF. Those with TET2-first mutations were inclined towards a diagnosis of ET and MF regarding 8 of 12 patients, with the residual balance of 4 patients diagnosed to PV. In the JAK2-first patients, the degree of homozygosity for the JAK2 V617F mutation in JAK2-first cultured cells is detected as 57%/C18% vs. 1%/C4% in TET2-first mutated cultured cells.

Given the overlap of above diagnoses regardless of mutational order, admittedly the timing of these specific mutations does not explicitly define the neoplasm. From the Darwinian perspective, conceivably there is a potential competitive advantage to clonal expansion of JAK2-first mutations from hematopoietic stem cells and manifested by greater phenotypic expression of PV [19,22]. This raises the distinct possibility that there are other somatic mutations beyond JAK2 and TET2 which drive these neoplasms and explain why ET can transition directly to MF rather than through PV. Candidate genetic alterations reported in association with PV for future study of mutational order are ASXL1 (12% associated frequency with PV) and SH2B3 (9% frequency) [23].

### Clinical presentation and diagnostic criteria

A plethora of symptoms are associated with PV including numbness of the extremities and tingling (acroparesthesias); burning pain, warmth and redness of the extremities (typifying the erythromelalgia of myeloproliferative disease); unexplained epigastric pain and weight loss; dyspnoea; visual and autonomic-like disturbances of sweating; vague symptoms of dizziness; and the disquieting pruritus associated with hot baths.

When generalized clinical symptoms of PV are evident, there are two characteristic profiles of the blood; (1) an increased red cell mass; and (2) associated blood hyper-viscosity. Classic hyper-viscosity syndrome of PV manifests as a triad of mucosal bleeding, visual disturbances and neurological symptoms. Acute obligatory therapy includes phlebotomy or erythrocytapheresis. The elevated viscosity levels at which patients become symptomatic are variable and measured by a viscosimeter in centipoise (cP), with a normal reading being less than 2.0 cP [24].

We have included the World Health Organization, WHO Diagnostic Criteria for Polycythaemia vera, 2016, Table 1 [1]. The original WHO 2008 inclusion criteria are revised, Hb > 18.5 g/dl in adult males lowered to >16.5 g/dl in adult females lowered to >16.0 g/dl [1,25,26]. The relevance of these modifications to major criteria 1 is that some cases of ET and unclassified MPNs with borderline Hb levels will be classified as having ‘masked’ or ‘prodromic’ PV [27]. Prodromic PV...
patients are at a substantially increased risk for progression to MF and AML compared to the well-defined PV; and younger patients may be at a higher risk of thrombosis [28]. As >70% of juveniles have negative JAK2 markers, their diagnosis of PV may remain elusive for some time, not yet satisfying enough major and minor criteria of the disease [2].

Regarding diagnostic criteria to measure total volume of erythrocytic ‘red cell mass’, there are comparative advantages and disadvantages of radiolabelling techniques that have been described [29]. For 51Cr-labelled erythrocytes, the RCM determinations are accurate to make a diagnosis of PV when relying upon experienced technicians. The problem surfaces that the 51Cr testing is complicated to perform estimated 2-6 times more expensive than simpler radio-iodine-labelled human serum albumin 125I technique and not always readily accessible to clinicians. Given these considerations, RCM determinations in adults may be unnecessary in cases of persistently elevated Hb and Hct levels above the diagnostic threshold.

### Differential diagnoses

#### Juveniles

Precision diagnosis and optimal treatment of PV demands careful attention to the clinical differential of disorders. True polycythaemia in neonates, children and juveniles needs to be differentiated from transitory elevated Hb in the neonatal period, twin-twin transfusion syndrome, maternal-foetal bleeds, trisomy syndromes, adrenal hyperplasia, erythropoietin secreting tumours, polycythaemia of the newborn seen with diabetic mothers and congenital causes of erythrocytosis [2].

The juvenile form of PV is distinguishable from juvenile ET with respect to platelet counts. The characteristic thrombocytosis for juveniles seen in ET presents at the mean, 1-109 × 10^9 l^-1 compared to the significantly lower counts seen in juvenile PV in the range 207–394 × 10^9 l^-1 [2]. With respect to platelet counts in adults, higher median counts of 811 × 10^9 l^-1 are reported in ET compared to 686 × 10^9 l^-1 seen in PV [30]. Across the broad spectrum of age for PV patients, these data suggest a general upward trend of the platelet levels between youth through adulthood. Platelet counts are logical biological markers to follow serially as possible indicators of disease progression.

### Adults

Extending the differential diagnoses to adult PV, the list includes chronic hypoxaemia related to COPD and heavy cigarette smoking; morbid obesity associated with chronic obstructive sleep apnoea; chronic habitation at high altitude; doping; various erythropoietin secreting tumours; states of blood volume contraction due to dehydration; autosomal dominant benign familial polycythaemia; recurrent bleeding states treated episodically by iron infusions and triggering exuberant RBC regeneration; middle age obese male hypertensive patients who smoke and are treated with diuretics, identified as Gaisbock syndrome or pseudo-polycythaemia; and patients with testosterone replacement therapy [31].

In adult patients, it is important to differentiate PV from ET otherwise clinicians may inadvertently apply phlebotomy to the latter group and cause unnecessary iron deficiencies. With respect to ET, it may distinguish itself from PV by manifest platelet counts in excess of 450 × 10^9 l^-1, bone marrow biopsy megakaryocyte proliferation and loose clusters, and positive molecular diagnostics of JAK2, calreticulin CALR and MPL proto-oncogene (thrombopoietin receptor mutations) [21]. Normal or elevated serum erythropoietin levels should discriminate out PV as the putative diagnosis. 51Chromium isotopic red cell mass determinations can differentiate ET from PV in adult patients, the latter having a higher risk for thrombosis and myelofibrosis complications [32].

### Table 1

| Major 1 criterion | Hb > 16.5 g/dl male; >16.0 g/dl female |
|-------------------|--------------------------------------|
| Major 1 criterion | or Hct > 49% adult male; >48% adult female; paediatrics** |
| Major 1 criterion | or red cell mass, RCM > 75% above the predicted value |
| Major 2 criterion | Positive genetic markers JAK2 V617F point mutation in exon 14 or various JAK2 mutations in exon 12 |
| Major 3 criterion | Hypercellular bone with proliferation of erythroid cells, megakaryocytes of different sizes and granulocytosis |
| Minor 1 criterion | A subnormal erythropoietin level |

*a. Two major criteria and one minor criterion are sufficient to diagnose PV.
**b. While not part of the WHO diagnostic criteria, which are specific to adults, in paediatric PV, reported are Hct criterion equivalent to 53–65% of the predicted value [12,58].

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**Vox Sanguinis** [2021]
Disease severity

Adult complications

Genetically, the evolution to aggressivity and complications of PV has been implicated to a loss of heterozygosity (LOH) on the short arm of chromosome 9 (9pLOH) [33]. This is likely because of a process of mitotic recombination in somatic cells and suggests a localized dominant mutation therein. The heterozygous form of the mutation is more prevalent and phenotypically less expressive, compared to the homozygous state. The explanation for this dichotomy of disease severity is rooted in the tenets of Mendelian genetics. The heterozygous form appears to compete with the wild type. A pairing of the mutated alleles in homozygosity amplifies gene expression phenotypically.

The onco-inflammatory response and bone marrow fibrosis

The ensuing development of MF in some adult PV patients is hypothesized to be linked to a chronic inflammatory milieu attendant to the co-existing neoplastic clones of cells, carrying mutations of JAK2. As the load of inflammatory driven reticulin fibrosis in the bone marrow escalates to grade 2, this pathology correlates to an increased risk of lymphoproliferative disease, AML [34]. An inflammatory cascade is triggered by various cytokines/chemokines released into the stromal microenvironments of the bone marrow and causal to fibrosis. When the neoplastic associated chronic inflammatory process is aborted after stem cell transplantation, there is noted a significant regression of bone marrow fibrosis associated with normalization of haematopoiesis [34].

Quantifiable blood levels of the inflammatory phase biomarker high sensitivity C-reactive protein, hs-CRP, have been reported to stratify the risk of thrombosis, haematological evolution and death in PV patients and those that transform to MF [34]. It is noteworthy that the JAK2 exon 14 and exon 12 mutations have an approximate equal probability of associated thrombotic events, fibrotic and leukaemic transformations, and overall survival [35,36]. The occurrence of mutations ASXL1 and bystanders SRSF2 and IDH2 are noted to negatively impact transformation-free survival of PV [23].

Paediatric complications

In the paediatric age group, an increased incidence of testing positive for JAK2 V617F has a greater association with thrombotic, haemorrhagic and other malignant conditions. This was reported in two smaller cohort studies.

In the first study of 11 patients followed serially in the ensuing decade of life after the diagnosis of paediatric PV, 27.3% of the individuals were JAK2 mutation positive but with zero reported thrombotic complications and deaths [2,12]. By comparison in a separate study of 36 paediatric PV patients, being JAK2 positive in 75% of the cohort, 37.5% of the patients collectively included antecedent malignancies; Budd–Chiari Syndrome (causal to hepatic vein stenosis/thrombosis, positive JAK2 V617F); stroke; gangrene; severe haemorrhage; and death [11,37,38].

Guidelines for phlebotomy, reduction of red cell mass in polycythaemia vera

Manual phlebotomy, first-line therapy

Manual phlebotomy merely requires intravenous access by a 16-gauge catheter and associated IV line, followed by drainage of whole blood in the range of 300–450 ml for adults as guided by body weight [39]. An even lower volume range of phlebotomy has been suggested but probably unnecessary because the risk of hypovolaemia is countered by the increased plasma volume and red cell mass of the disease [39].

The typical length of the manual phlebotomy procedure is 10–15 min, though patients are often asked to stay in the infusion clinic for observation to ensure their hemodynamic stability for some time afterwards. It is appropriate to request the patient to consume a cumulative total of one litre of liquids taken orally, divided equally before and after phlebotomy. Initially, the induction treatment frequency is typically every one to two weeks, though may be escalated to twice weekly, and maintenance extended to monthly or even less frequently for some patients to target a Hct of less than 45% [39,40]. For patients presenting with recalcitrant hyper-viscosity syndrome, a lowered Hct in the 40–42% range would be an appropriate intervention [39].

As each manual phlebotomy session decreases the Hct by around 3%, multiple sessions are required to reach a target Hct of less than 45%. It is imperative to titrate the Hct consistently because a chronically high Hct is associated with an increased risk of thrombotic complications [41]. Twenty-five per cent of patients find the repetitive phlebotomies uncomfortable and inconvenient, and as a result, there is the potential for non-compliance, which is observed in up to 8% of PV patients [42]. Though single phlebotomies are estimated to be nearly 71% less expensive compared to single procedure erythrocytapheresis (ECP—not to be confused with extracorporeal photopheresis), proponents of ECP suggest that an overall reduced treatment frequency compensates to equalize costs of the
two modalities [43]. Nonetheless, the majority of red cell depletion procedures for PV continue to employ manual phlebotomy, whereas ECP is relegated to special circumstances.

Large volume isovolaemic erythrocytapheresis, first-line therapy

Erythrocytapheresis is an extracorporeal centrifugation driven technology, primarily intended to reduce red cell mass in patients with PV to normal levels and secondarily to separate red blood cells from whole blood in blood banks. In practice, ECP can be considered an extension of whole blood component preparation by the routine ‘bottom and top’ technology. Today, preparation by various apheresis technologies is utilized in practice to ensure the highest standards of safety/efficacy and reliability while employing modern automated equipment.

The practicability of ECP is enumerated in Table 2, and there are supportive evidence-based guidelines [5]. Two clinically relevant scenarios qualify for a trial of ECP. (1.) A trial of ECP is indicated for patients who consistently require increased frequency of manual phlebotomy to avoid or delay chemotherapy. Nonetheless, ECP would be excluded, non-indicated in those patients having a known phenomenon of temporary phlebotomy resistance (defined rather conservatively as >=3 manual phlebotomies per year transiently) [39]. (2.) In order to prevent intraoperative/perioperative vascular complications of emergent surgery, ECP takes precedence to achieve an Hct of less than 45% in a single treatment session [39].

After receiving ECP treatment, patients experience a rapid reduction in RBCs and WBCs, though platelet counts tend to remain relatively unchanged [42]. After decreasing red cell mass, the resulting iron deficiency does not generally warrant intervention unless severe with coincidental symptoms, because doing so would unfortunately trigger the iron-dependent proliferation of erythropoietic precursor cells and thus reinvigorating red cell production [39,44].

Risk stratification of disease guiding cytoreductive pharmacotherapy

Low-risk disease

These patients are typically less than age 60, have no history of arterial venous thrombosis, but may have some uncontrolled microvascular features of disease such as numbness, tingling, erythromelalgia, pruritus; cardiovascular conditions including hypertension; or haematological aberrations, that is leukocytosis [21]. Consistent with controlled studies, low dose aspirin, ASA 81mgs once or twice a day should be sufficient to reduce thrombotic risk [45]. The decision to treat once or twice daily with low dose ASA is predicated on how well the microvascular symptoms are controlled and the plus/minus presence of comorbidities, that is hypertension and leukocytosis. A double dose of mini-dose ASA to sufficiently suppress thromboxane A2 synthesis is indicated to interdict elevated platelet aggregation in MPN disorders [46].

High-risk disease

Such patients are ≥60 years of age and JAK2 mutation positivity or a history of arterial venous thrombosis [21]. The initiation of pharmacological cytoreduction intervention is a key note of these high-risk patients for which hydroxyurea is a prime candidate. Comparable to the low-risk stratification, mini-dose ASA is indicated in single or double dose forms daily because of the drug’s anti-thrombotic properties [21]. Anti-coagulation would be indicated for concomitant deep venous thrombosis of the extremities, pulmonary embolism and thrombotic complications of the liver (Budd–Chiari syndrome). A lytic drug would have value for treating untoward acute arterial strokes guided by medical protocols of administration.

For the high-risk patients, below are characterization of the major benefits and some drawbacks of the recommended first or second-line drugs. We feature the principal role of hydroxyurea, a favourite choice for first-line cytoreductive therapy and busulfan, peginterferon alpha-2a and ruxolitinib as second-line agents.

Hydroxyurea: first-line

Hydroxyurea (HU) also known as hydroxycarbamide (HC) is a cytotoxic antimetabolite with the potential to cause severe myelosuppression. As an older but favoured first-line drug, it is used as an effective cytoreductive agent in the treatment of PV. Based upon tissue culture studies in humans and rats, its principal mode of action is thought to be as a ribonucleotide reductase inhibitor and causing immediate pronounced cessation of DNA synthesis [47].

In the Spanish Registry of Polycythaemia Vera opened in 2011, 890 patients with a history of receiving HC (aka HU) cytoreductive therapy were tracked using the European LeukaemiaNet (ELN) criteria [3]. This included categorization of clinico-haematological response, resistance and intolerance to the drug. Tracking criteria included measuring Hct, Plt and WBC in response to HC therapy while compared to defined laboratory threshold values; need for phlebotomy; symptomatic or excess splenomegaly; uncontrolled myeloproliferation; cytopenias; and extra-haematological toxicities. In this sizeable study of PV, 78% of patients received a complete or partial clinical
Table 2 Therapeutic erythrocytapheresis: practicable utility in selective polycythaemia vera patients.

| Approach                                                                 | Practicality | Outcome |
|--------------------------------------------------------------------------|--------------|---------|
| First-line therapeutic option, strong grade I recommendation by the ASFA to reduce the red cell mass effectively [5]. Rapid normalization of the Hct in order to treat or prevent life threatening pro-thrombotic or haemorrhagic complications; and in preparation for emergency surgery [39]. | Practicable | First-line therapeutic option recommended by the ASFA to reduce red cell mass effectively. |
| Capacity to achieve pronounced red cell extraction with a single session to achieve Hct < 45% | Practicable | Achieve pronounced red cell extraction with a single session to achieve Hct < 45% |
| Technical notes: a. Using automated ECP, the red cells are depleted after apheresis and re-infusion of the plasma, buffy coat cells, and additional crystalloids, ie. isotonic saline and albumin [60]; b. The volume of red blood cells Vₘ to be removed by ECP can be reasonably estimated by the following formula [44,59]. c. ECP-erythrocytapheresis; d. ASFA-American Society for Apheresis. VR = \frac{\text{desiredHCT}}{\text{initialHCT}} \times \frac{\text{bloodvolumein} \, mL}{\text{bodywtin} \, kg} | - | - |

response and 22% no response. By at least one of the ELN criteria, 15-45% of the total patient series were deemed to be resistant or intolerant to the drug or both. Given the non-responders or those with resistance and/or intolerance, consideration of second-line drug therapy such as busulphan, peginterferon alpha-2a and ruxolitinib is in order.

**Busulphan: second line**

Heretofore, the primary experience to treat PV with busulphan has been by first-line therapy orally. Busulphan was recently tested as a second-line cytoreductive agent to treat HU intolerant or resistant PV and ET patients. Durable haematological responses were achievable in 75% of the combined patient pool, being able to discontinue busulphan. Partial reductions in molecular JAK2 V617F allele burdens were observed in 33% of the tested patients [48].

Busulphan is a bifunctional alkylating agent, possessing intense myeloablative activity with associated mutagenic, clastogenic and cytotoxic effects. The pharmaceutical activity of the drug is propagated by dual methyl sulphoxonates on a short alkyl chain that are hydrolysed to carboxyl ions. These alkylate the DNA non-specifically in the cell cycle resulting in a configuration of DNA rearrangements, fragmentation, cross-linked guanine bases, inhibition of DNA double helix uncoiling, miscoding and inhibition of replication and transcription [49,50]. Inherent to this cocktail of antagonistic actions to DNA, busulphan possesses dose-dependent bone marrow suppression, which requires vigilance. Despite being a DNA alkylator, busulphan has not been well correlated to leukaemic transformation, and if there is risk, it appears to be relatively low, a plus for use of this drug as a therapeutic [21,51].

**Peginterferon alpha-2a: second line**

Recombinant peginterferon alpha-2a’s (peg INF alpha-2a) mechanism of action includes binding to type 1 human interferon receptor, receptor dimerization, activation of the JAK/STAT pathway and having downstream pleiotropic effects on multiple cell types. Peg INF alpha-2a has the capacity to produce pronounced neutropaenia, lymphocytopenia, RBC anaemia and thrombocytopenia [52]. It is recognized as a second-line cytoreductive agent in the treatment of PV patients that are refractory or intolerant of HU [21].

The drug has capacity to induce partial molecular remissions of JAK2 mutation burdens (but of debatable clinical import) and observable durable haematological remissions. Therapy alleviates burdensome splenomegaly and pruritus but managing the drug is delicate, dealing with a miscellany of toxic side-effects that relegate it as a second-line agent [21].

**Ruxolitinib: second line**

The first licensed ATP dual competitive JAK1/JAK2 inhibitor is ruxolitinib (RUX), which became FDA approved in 2014. The drug is part of the class of compounds known as tyrosine kinase inhibitors (TKIs). Importantly, there are two common misconceptions about the mechanism of action with respect to the binding of this drug to the JAK1/JAK2 tyrosine kinases. Errorneously, it is thought the kinase needs to be mutated to bind the drug and that the drug complexes to the actual mutated JAK2 V617F site. As points of edification, RUX complexes with JAK kinases, but is bound to the JAK2 ATP/catalytic pocket, not the site of mutation [53]. In the treatment of PV, its principal pharmaceutical activity occurs as an inhibitor of the catalytic site of the protein which
overdrives the production of myeloid cell lines. RUX clearly does not markedly improve allele burden of mutant \textit{JAK2}, nor can it reverse bone marrow histopathology [14].

The Janus kinase 1 gene is crucial to the expression of genes that mediate the inflammatory response, and Janus kinase 2 is essential to cytokine and growth factor signalling [18,54]. The partial pharmacological inhibition of JAK1 and JAK2 protein catalytic sites by RUX is valuable as maintenance therapy to mitigate disease severity yet has profound consequences given the crucial roles of the parent genes. Patients are at heightened risk for opportunistic infections, cytopenias, bleeding and neurological sequelae [55]. If these complications ensue, the clinician must be well attuned to immediately identify them and consideration to lower the dosage of RUX or discontinue it.

\textbf{Evolution of genetic resistance to tyrosine kinase inhibitors, the molecular biology}

The mechanism by which patients become refractory to RUX is of great interest because it has introduced novel kinase inhibitors thought to have activity in the treatment of PV transformation to myelofibrosis.

The following research was supported by one of our contributors, Azam [56]. In an in vitro model of drug-resistant screening using a murine cell line BaF3, it expressed randomly mutagenized \textit{JAK2-V617F} proteins followed by selection with \textit{JAK2} inhibitors RUX or fedratinib [56]. Subsequent analysis identified 211 drug-resistant mutations against RUX clustered in kinase and regulatory domains of \textit{JAK2} (Fig. 2). These mutational hot spots conferred resistance not only to RUX but also showed cross resistance to other \textit{JAK2} inhibitors AZD1480, CYT-387 and lestaurtinib [56]. However, there is no observation of resistant clones against fedratinib. Perhaps more interestingly, all RUX resistant mutations were fully sensitive to fedratinib suggesting a unique mechanism of kinase inhibition which can efficiently suppress genetic resistance.

Further, enzymatic and structural studies revealed that fedratinib binds two different sites in the kinase domain in proximity: (1) ATP-binding and (2) peptide-substrate binding sites. So far, anti-kinase therapy exploits the ATP-binding site for drug targeting which is prone to develop resistance over the course of treatment. Mechanistically, mutations in the ATP-binding sites in the kinases including \textit{JAK2} prevent drug binding without affecting the ATP-binding and catalytic activity, thus conferring drug resistance. In contrast, mutations in the substrate binding pocket kill the kinase activity. To confer drug resistance, the kinase must be catalytically active; therefore, drug-resistant mutations in the substrate binding pocket failed to emerge as these inactivated the kinase [56]. This study provides a proof of concept to target the substrate binding pocket to design resistance free kinase inhibitors. These novel types of TKI inhibitors are being tested in the treatment of myelofibrosis and, in particular, may have application to post-PV transformation [57].

\textbf{Summary}

With respect to the origination of PV, we highlight the principal discovery of the \textit{JAK2 V617F} mutation in a phosphorylating JAK2 kinase protein. The mutation is consequent to a dominant point mutation in the parent Janus kinase 2 gene and phenotypically is most expressive in its homozygous form. These genetic errors translate to
mutations in the JAK2 kinase protein causing uncontrolled gain in activity and excess red cell production.

Disease transformations have been observed in patients followed in excess of 10 years, between ET, PV and MF, the transitions occurring sequentially. Yet the MPN diseases are not always so orderly and paradoxically, some patients matriculate directly from ET to MF directly. The possible explanations for this diversity of disease behaviour include such factors as progressively increasing allele burden of genetic mutations and timing or order of specific mutations.

It is rather important that clinicians be cognisant of the complex differential diagnoses of PV. The WHO provides inclusive criteria of a lower Hb threshold of 16.0–16.5 g/dl, not to miss the diagnosis of PV in a ‘pro-dromic’ group of patients known to be at higher risk for leukemic transformation.

Polycythaemia vera can be stratified into low- and high-risk patients, the latter being age defined ≥60 JAK2 positive or those with a history of thrombosis. While phlebotomy and low dose ASA are front-line treatments for low-risk patients, chemo-cytoreductive therapy and ASA are introduced in the high-risk class to prevent thrombosis.

While more than five decades old, hydroxyurea by virtue of its cytopreductive activity has proven to be an essential drug in the treatment of PV. In refractory patients, busulphan and peg INF alpha-2a are good second-line alternatives as cytoreductive agents. RUX, a JAK2 kinase inhibitor, has a relatively smaller role in the maintenance treatment of PV. Novel TKI drugs are being explored (1) to suppress drug resistance and (2) assess their potential therapeutic application in post-PV, when it transforms to MF.

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Conflict of interest

None to report.

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