Literature Review

Polycythemia Vera

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Polycythemia vera is a rare, acquired clonal disorder of the hematopoietic stem cells resulting in unregulated proliferation of erythropoiesis leading to an accumulation of red cells. The main complications of the disorder arise from hyperviscosity and an increased risk of thrombosis. In recent years, advances in next generation sequencing (NGS) techniques have identified promising targets to enable more accurate risk stratification of patients as well as targets for new classes of therapeutic drugs. This article aims to review the current research in this field to provide an overview of the key features of this disease.

Key words: Polycythemia vera, myeloproliferative neoplasms, erythrocytosis

Introduction

Polycythemia vera (PV) is an acquired clonal disorder of the hematopoietic stem cells.[1] It is the most common of the Philadelphia negative myeloproliferative neoplasms (MPNs), arising due to a loss of the regulatory mechanisms for erythropoiesis, resulting in the overproduction of red blood cells (RBCs).[2,3] First described in 1892, the discovery of janus kinase 2 (JAK2V617F) in 2005 has revolutionized the diagnosis of this condition. This, paired with technological advances in NGS and the therapeutic use of monoclonal antibody-based drugs, has stimulated an explosion of active research in recent years.

Epidemiology

PV has an incidence of 0.4-2.8 per 100,000 persons/year, occurring in 1/3300 people worldwide.[1] It can occur at any age but is rare in those under 60 years of age.[1,2] Under the age of 60 it is more commonly diagnosed in women, but this equalizes in the over 60 age bracket.[3] However, there is some controversy regarding the gender ratio, with some reporting a lower incidence in women overall.[4] This discrepancy in the reported gender ratio may be due to rapid changes in diagnostic criteria.[2] For most patients, PV will present as an indolent disease and if treated people may live with the disease for more than 40 years. However, PV is thought to be underdiagnosed, as it is frequently mistaken for essential thrombocythemia (ET) and for this class of patients, the lack of appropriate treatment may lead to a worse prognosis.[3,5]

Pathogenesis

The pathogenesis of thrombosis as seen in PV is multifactorial and complex, arising from the interplay between different cell lineages. In red blood cells (RBCs), the constitutive activation of the JAK-STAT (janus tyrosine kinase signal transducer and activator of transcription) pathway due to the JAK2V617 mutation (found in 95% of PV patients) leads to phosphorylation of Lu/BCAM (Lutheran blood group and basal cell adhesion molecule), resulting in abnormal adhesion to the subendothelial protein laminin.[6-8] Meanwhile, the increased neutrophil count results in increased proteolytic enzyme

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(elastase and cathepsin G) activity as well as an increase in reactive oxygen species. This is accompanied by an increase in the expression of CD11b (pan-macrophage marker), leading to increased platelet activation and endothelial damage, with the subsequent increased release of tissue factor.[8] At the same time, there is often an increased platelet count with increased platelet activation leading to an increase in platelet induced thrombin production. This is worse in immature platelets. The increase in immature platelets correlates with the JAK2V617F allele burden.[3] The activated platelets also show increased expression of P selectin (cell adhesion protein) on their surface.[8] When paired with the reduced blood flow and endothelial damage as a result of hyperviscosity, these events combine to produce a perfect storm of increased procoagulant and proteolytic properties, with increased secretion of inflammatory cytokines and increased expression of adhesion molecules.[8] In contrast to the procoagulant effects of PV, extreme thrombocytosis (platelet count >1500 x10 ^{9}/L) may be associated with an increased risk of hemorrhage due to an acquired von Willebrand syndrome (VWS).[5] The exact mechanisms underlying fibrotic and leukemic transformation of PV are poorly understood, but evidence suggests that aging is associated with the accumulation of potentially harmful somatic mutations.[6]

Clinical and Diagnostic Findings

The classical presentation of PV (Table 1) is erythrocytosis in isolation or combination with leukocytosis and/or thrombocytosis.[3,5,8,9] PV occurs in both indolent and aggressive forms, with the more indolent forms often being identified only as an incidental finding.[5] In contrast, more aggressive forms may lead to symptoms which significantly reduce quality of life.[5] Females tend to have fewer deregulated genes and thus may present with a less severe clinical picture than males.[4]

An increased hematocrit may be the first indication of PV, with the hematocrit thought to be more important in the diagnosis than hemoglobin, because hyperviscosity results from increased RBC numbers rather than individual RBC content.[3,9] Therefore, patients with a persistently increased hematocrit (above 0.52 in males and 0.48 in females) will warrant further investigation.[9] Early detection of PV presents challenges as the increase in hematocrit will often be matched by an increase in plasma volume, thus masking the overall increase in red cell mass. In cases where the hematocrit is less than 0.590, it will be difficult to differentiate between PV and other causes of erythrocytosis (Table 2).[3] This plasma expansion means that, in contrast to the World Health Organization's 2016 guidelines (Table 3) PV cannot be excluded based on a normal hematocrit alone.[5-9] The situation is further complicated by conditions such as pregnancy or

Table 1. Complications and symptoms of Polycythaemia vera

Complications	Symptoms
• Splenomegaly due to extramedullary hematopoiesis	• Gastric discomfort • Early satiety
• Increased secretion of proinflammatory cytokines	• Pruritus
• Hyperviscosity	• Occipital migraines • Dizziness • Erythromelalgia • Amaurosis fugax • Transient Ischaemic Attacks (TIA)
• Arterial and venous thrombotic events	• Pain, swelling, warmth, redness, and cramps particularly in the legs (DVT) • Increased incidence of miscarriage • Chest pain, shortness of breath (pulmonary embolism, myocardial infarction) • Increased incidence of strokes/TIA
• Hemorrhage	• Epistaxis
• Hyperuricemia	• Severe pain and stiffness of the joints • Red, swollen and or misshapen joints

Key: DVT- deep vein thrombosis.

Table 2. Exclusion of causes of secondary Erythrocytosis

Cause	Diagnostic Test
Drugs, smoking, alcohol	• Comprehensive patient history with systematic questioning
Renal tumours and hepatic disease	• Urea and Electrolytes, • Liver Function Tests including serum calcium • Erythropoietin levels (raised) • Ultrasound
Tissue hypoxia	• Arterial oxygen saturation (May be misleading in cases of carbon monoxide poisoning, high affinity hemoglobins and sleep apnoea) • Erythropoietin levels (raised)
Dehydration	• Red cell mass
Congenital causes such as mutations of the erythropoietin receptor genes	• Family history • Gene sequencing
High affinity hemoglobins	• HPLC • Mass spectrometry • Gene sequencing

Key: HPLC- High pressure liquid chromatography.

Table 3. Diagnostic criteria for Polycythemia vera

• RBC mass 25% above the mean normal predicted value
• Hemoglobin > 165 g/L in men and >160 g/L in women
• Hematocrit >0.490 in men and >0.480 in women
• Trilineage hypercellularity in the bone marrow
• JAK2v617F or JAK2 exon 12 mutation present
• Subnormal serum erythropoietin

Key: RBC- Red blood cell.

splenomegaly, which will also cause plasma expansion. The hematocrit may also be normal in cases of iron deficient PV, as the body defends the mean cell hemoglobin concentration (MCHC) by reducing the mean cell volume (MCV). Therefore, an isolated increased red blood cell count may be the only suspicious finding .[3] This presents a cautionary tale against the practice of offering a trial course of iron supplementation for unexplained microcytosis. It should be noted that ferritin levels are often low in iron deficient polycythemia as the diminished iron stores limit erythropoiesis.[9] However, the additional presence of leukocytosis with or without splenomegaly may help to secure the diagnosis.[3] Ultrasound may be useful to detect splenomegaly in the absence of a palpable spleen and can also be useful for the detection of renal and/or hepatic pathologies. The diagnosis is further complicated by the fact that MPNs are not mutually exclusive. The systematic exclusion of secondary causes of erythrocytosis (Table 2) is of paramount importance.[3]

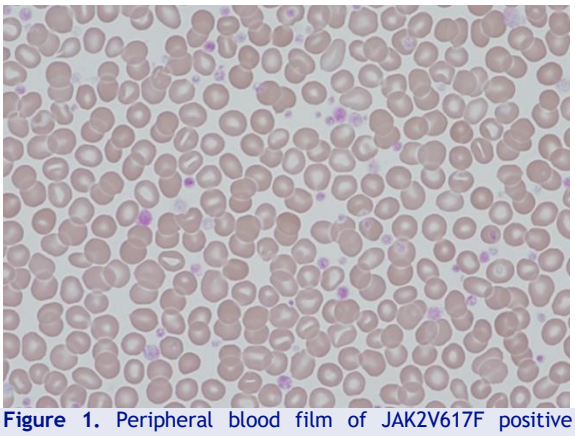

Figure 1. Peripheral blood film of JAK2V617F positive polycythemia vera at x600 magnification stained with May Grünwald Giemsa. Note the thrombocytosis and platelet anisocytosis that may result in a misdiagnosis of essential thrombocythemia.

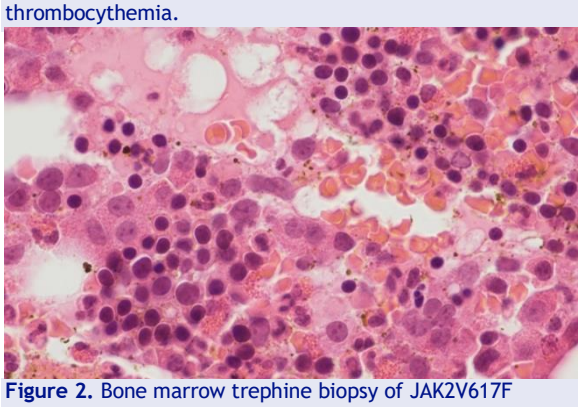

Figure 2. Bone marrow trephine biopsy of JAK2V617F positive polycythemia vera at x600 magnification stained with hematoxylin and eosin. Note the expanded erythroid islands

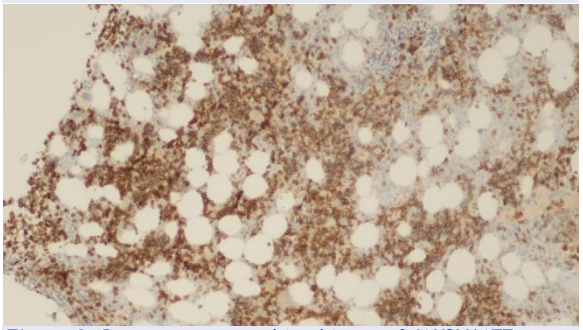

Figure 3. Bone marrow trephine biopsy of JAK2V617F positive polycythemia vera using Glycophorin-C immunohistochemical staining at x100 magnification. Note erythroid hyperplasia and expanded erythroid islands

The complete blood count (CBC) can confirm a persistently raised hematocrit along with the presence of leukocytosis and/or thrombocytosis (Table 3).[5-9] The subsequent peripheral blood film (Figure 1) may then identify circulating blasts or leuko-erythroblastic features, which may be indicative of bone marrow impairment, triggering a bone marrow biopsy.[9] The bone marrow biopsy can be useful for distinguishing between PV and ET (Table 4), demonstrating significantly increased erythroid precursors relative to the other myeloid precursors in the case of PV (Figures 2 and 3).[7-9] The other myeloid precursors may be moderately increased and left shifted and there may be anisocytosis of the megakaryocytes with larger forms seen more frequently, which may display uneven or reduced lobulation. However, several studies have reported a failure to reach a diagnostic consensus based on morphological findings alone.

Table 4. Differential diagnosis of Polycythemia vera relative to Essential Thrombocythemia

• Lower platelet count
• Lower MCV
• Lower ferritin
• Lower erythropoietin levels
• Increased splenomegaly
• Increased pruritus

Key: MCV- Mean cell volume.

A direct measurement of RBC mass and plasma volume affords a more concrete diagnosis and alongside erythropoietin levels and arterial blood gas analysis, this was the most used diagnostic test for PV in 2002.[1,3] However, in the post JAK2V617F era, this is rarely performed due to the human and financial resources required, which are often prohibitive.[1,9] The detection of JAK2V617F remains the cornerstone of diagnosis for PV and will usually be used in conjunction with hemoglobin as a surrogate for RBC mass.[9] It is important to note that the mutation will frequently also be seen in the other MPNs (Table 4) and although the neutrophil JAK2 allele burden tends to be higher in PV than in ET, a valid threshold has not been established.[3,7-9] It should also be noted that this mutation has been identified at low levels in normal patients, with the allele burden increasing with age.[3,9]

Prognosis and Risk Stratification

The median age of PV patients at diagnosis is 61 with a median survival of 18.9 years, ranging from 10.9 to 27.8 years depending on the risk group.[1] Potentially fatal complications for PV include fibrotic (15% of patients) and leukemic transformations (1.5% of patients), both of which are associated with a significant exacerbation of symptoms and reduced life expectancy, with a median survival of 1.5-2.5 months untreated.[3,8,10] Therefore, transformation carries a poor prognosis and treatment is challenging.[10] However, it should be noted that post PV myelofibrosis has a more favorable prognosis than de-novo primary myelofibrosis.[3] Post PV acute myeloid leukemia typically has a French American British Classification (FAB) M6 or M7 phenotype and is relatively resistant to chemotherapy, with an allogeneic stem cell transplant being the only curative option, as most drugs are ineffective at securing a long-term remission.[11] Transformations, if they occur, will usually occur within 12 years of the initial diagnosis. Transformation from ET to PV also occurs in 20-30% of JAK2V617F positive cases and is more commonly seen in women.[3] The most common cause of morbidity and mortality in PV patients is thrombotic events. Traditional risk factors for thrombosis such as smoking, diabetes mellitus

Table 5. International working group for MPN research and treatment (IWGMRT) prognostic scoring system

Risk Factor	Weighed Hazard Ratio Points	Points	Risk Stratification	Median Survival (years)
Age		0	Low	26
• ≥ 67 years	5			
• 57-66 years	2			
Leukocytosis		1-2	Intermediate	15
• WBC > 15 x10^9/L	1			
Previous thrombotic event	1	≥ 3	High	8.3

Key: WBC-White blood cell count.

and hypertension, which are not currently included in available prognostic systems, should also be considered in any prognostic evaluation of patients.[11,13] Based on this consideration, some low risk patients may be regarded as high risk due to pre-existing cardiovascular disease or cardiovascular risk factors.[8,9]

Existing tools for the risk stratification of patients with PV include the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWGMRT) (Table 5), the Mutation Enhanced Prognostic Scoring System (MEPSS) (Table 6), and Dynamic Prognostic Model. [7-12] The IWGMRT prognostic score has not yet been validated by prospective studies. However, the Dynamic Prognostic Model can be useful in monitoring the impact of treatment and adjusting care plans accordingly. Risk factors used by this model are associated with a 4.2-fold increase in the risk of death and are defined as a hemoglobin less than 100 g/L, a platelet count less than 100 x10^9/L and a white blood cell count (WBC) greater than 30 x10^9/L.[9] The low frequency of PV combined with an even lower frequency of abnormal karyotypes has provided little data on which to evaluate the prognostic potential of cytogenetic abnormalities, but it may be useful to obtain cytogenetic information at diagnosis and following changes in the clinical course of the disease.[8,12] More recent candidates for prognostic evaluation include both driver mutations (directly responsible for dysregulated cellular proliferation) and non-driver mutations (which exacerbate the complications of the disease but do not directly affect proliferation). Active research continues in an attempt to establish the role of the mutations in the pathophysiology of PV and thus their prognostic value (Tables 7, 8a and 8b). [7-9,14,15]

Table 6. Mutation enhanced prognostic scoring system (MEPSS)

Risk factor	Fibrotic transformation	Leukemic transformation
≥60 years of age	✓	✓
WBC 15-30 x10^9/L	✓	✓
Homozygous JAK2 mutation	✓	
Exposure to alkylating agents	✓	✓
Previous radiotherapy		✓
Non-driver mutations of myeloid genes including ASXL1, SRSF2, RUNX1, SF3B1, IDH1/2	✓	✓
TP53 mutations		✓
Reticulin fibrosis and raised LDH	✓	
Splenomegaly	✓	✓
Abnormal Karyotype (+1q, +8, +9, 20q- most associated with transformation)		✓

Key: LDH – Lactate dehydrogenase, WBC-White blood cell count.

In addition to the association with increased risks of fibrotic and leukemic transformation of PV, DNA-methyl transferase 3 alpha (DNMT3A), tet-methylcytosine dioxygenase 2 (TET2) and ASXL transcription regulator 1 (ASXL1) mutations, collectively referred to as DTA mutations, have also been associated with increased thrombotic risks.[13-15] DNMT3A, TET2 and ASXLI are epigenetic regulators. Mutation of these genes leads to altered DNA methylation, resulting in increased transcription of proinflammatory genes. This results in increased secretion of inflammatory cytokines, such as interleukin 1ß. The increase in inflammatory cytokines predisposes patients to inflammatory conditions such as atherosclerosis, resulting in an increased risk of thrombotic and thrombo-occlusive events. [13]

NGS for somatic mutations is not yet standard practice, but it may become so as technological advances make this option more accessible, as inclusion of this information has been shown to improve the accuracy of prognostic assessment.[9] Improved knowledge and application of DNA analysis may improve risk stratification of patients for both transformation and thrombotic events.[7] Therefore, if the predictive value of these mutations can be verified, it may enable the identification of patients requiring closer monitoring or a more proactive treatment plan, including anti-inflammatory agents for example.[13,14]

However, JAK2V617F neutrophil allele burden remains the main stay for staging of the disease.[3] This has the advantage of being able

Table 7: The prognostic impact of somatic driver mutations

Driver mutations	Function	Prognostic impact of mutation
JAK2V617F	• Constitutively active tyrosine kinase	• Homozygosity is linked to reduced platelet count, increased splenomegaly, increased need for cytoreductive therapy and increased pruritus. • An allele burden over 50% is associated with increased risk of fibrotic transformation but not leukemic transformation. • An allele burden of less than 50% is indicative of the indolent form of the disease,
JAK2V625F	• Tyrosine kinase with enhanced function	• Under investigation
JAK2 F556V	• Tyrosine kinase with enhanced function	• Under investigation
CALR	• Multifunctional Ca^{2+} binding endoplasmic reticulum chaperone protein. • Binds and activates MPL in a TPO dependent manner.	• More commonly seen in males and in younger patients. • Associated with a reduced risk of thrombosis relative to JAK2V167F positive patients
CXCL4/PF4	• Inhibition of cell death due to downregulation of TGFB signalling	• Under investigation

Key: JAK- Janus Kinase, MPL- Myeloproliferative leukemia, also known as thrombopoietin receptor gene, TPO-Thrombopoietin, TGFB – Transforming Growth Factor Beta, CXCL4/PF4 - Platelet factor 4.

Table 8a: The prognostic impact of somatic non-driver mutations

Non-Driver mutations	Function	Prognostic impact of mutations
TP53	• Encodes p53 tumour suppressor protein essential for DNA repair. • Inactivation leads to increased hematopoietic stem cell self-renewal and resistance to cellular stress	• Associated with cytopenia following hydroxyurea therapy. • Increased risk of leukemic transformation • Poor prognosis for overall survival
RUNX1	• Encodes a transcription factor involved in hematopoiesis. • Inactivation leads to reduced myeloid differentiation and increased hematopoietic stem cell self-renewal	• Genetic instability • Increased risk of leukemic transformation. • Reduced overall survival
SRSF2, ZRSF2, U2AF1, SF3B1	• Splicing factors which play an important role in DNA stability	• Increased risk of acquiring further mutations. • Associated with cytopenia following hydroxyurea therapy • Increased risk of fibrotic and leukemic transformation. • Reduced overall survival rate
TET2	• Encodes enzyme that catalyses conversion of 5-methyl cytosine to 5 hydroxy methyl cytosine resulting in DNA methylation • Inhibition of TET2 leads to decreased DNA methylation and impaired hematopoietic differentiation • Epigenetic repression of tumor suppressor genes • Increased expression of hematopoietic stem cell self-renewal genes	• Increased risk of fibrotic and leukemic transformation. • Associated with increased risk of vascular events. • Reduced overall survival rate

Table 8b. The prognostic impact of somatic non-driver mutations (continued)

Non-Driver mutations	Function	Prognostic impact of mutations
IDH1/2	• Encode enzymes that catalyse conversion of isocitrate to ketoglutarate which acts on TET2. • Important for protection from oxidative stress	• Associated with cytopenia following hydroxyurea therapy. • Increased risk of fibrotic and leukemic transformation • Reduced overall survival rate
ASXL1	• Encodes a nuclear polycomb protein that affects regulation of transcription and RAR mediated signalling. • Interacts with chromatin modifying proteins including PCRC2	• Associated with increased WBC and platelet counts. • Increased risk of fibrotic and leukemic transformation • Reduced overall survival rate. • More likely to be transfusion dependent.
LNK/SH2B2	• Inhibits signalling through tyrosine kinase receptors such as the erythropoietin receptor. LNK mutations disrupt negative feedback loops affecting proliferation.	• Associated with increased extramedullary hematopoiesis and enhanced growth of JAK2V167F positive cells in clonal assays and mouse models.

Key: WBC – White blood cell count

to be performed on peripheral blood samples. In terms of monitoring, there are no recommendations to monitor the allele burden sequentially, as the clinical impact of directly lowering the allele burden has yet to be confirmed.[3,9,14] Similarly, there are no indications for serial bone marrow biopsies to monitor morphology or fibrosis, but a repeat bone marrow biopsy may prove useful if transformation is suspected.[9]

Treatment

A typical treatment pathway for PV usually begins with phlebotomy and low dose aspirin, prior to risk stratification. High risk patients will then go on to receive cytoreductive therapies such as hydroxyurea (HU) or interferon as a first line therapy.[16] Ruxolitinib may then be used as second line therapy, with subsequent leukemic transformations treated with chemotherapy and/or allogeneic stem cell transplants for suitable candidates.[16]

Treatment of PV focuses on minimising the risk of thrombosis, reducing myeloproliferation, alleviating the symptoms of the disease and managing the complications (Table 1).[7,16] Treatment may yield a symptomatic, hematological, or molecular response which may be complete or partial. In contrast if there is no response, the disease will be progressive. The impact of a complete hematological response on long term survival, including thrombotic risk and disease progression, has yet to be fully established.[7]

A monthly schedule of phlebotomy is the cornerstone of treatment in low risk cases.[5,17] It reduces the red blood cell mass and expands the plasma volume.[5] The link between reducing the hematocrit and reducing the thrombotic risk was established by the CYTO-PV trial.[16] However, the same study failed to establish a link between reducing leukocytosis and thrombotic risk.[16] Phlebotomy is generally combined with low dose aspirin, although recent studies have cast doubts on the benefits of the prophylactic use of aspirin in asymptomatic patients without additional risk factors for cardiovascular disease.[3,10]

A consensus target of a hematocrit of 0.45 independent of gender has been established, based on the association with increased cerebral blood flow and reduced risk of vascular occlusive episodes. However, there have been no trials which confirm this effect with phlebotomy in isolation, with most study cohorts including a significant proportion of patients who were also being treated with HU.[17] There are also recommendations for a target below 0.42 for patients with persistent or recurrent symptoms.[1,2] In addition, despite a lack of evidence to support gender specific targets, such targets are applied by almost half of clinicians. In such cases a lower threshold of 0.42 is generally applied for women.[9] There are conflicting opinions on the application of lower thresholds during pregnancy, with some

advocating a target below 0.33.[5,8,17] This may be more appropriate in high risk pregnancies.[17] Phlebotomy may alleviate symptoms associated with hyperviscosity but is not effective at treating severe headaches or pruritis.[5]

Apheresis is an additional option for reducing the haematocrit and has the advantage of being able to achieve the target hematocrit in one session.[17] However possible side effects include dizziness, twitching, muscle cramps, fainting, arrhythmias and fever. It is also very expensive when compared to phlebotomy. It may, however, be an appropriate option where a rapid reduction in the hematocrit is necessary, e.g. to reduce the risk of thrombosis prior to emergency surgery.[17]

Cytoreductive therapies such as HU may be indicated for higher risk patients. A platelet count greater than 1000 $x10^9$/L may be used as a trigger for the implementation of this therapy, but many clinicians will only treat symptomatic thrombocytosis, as concerns that post phlebotomy thrombocytosis may increase thrombotic risk.[1,17] Reducing thrombocytosis may relieve migraines and reduce transient ischemic attacks. As previously discussed, extreme thrombosis may be associated with von Willebrand like syndrome, which occurs due to excess platelets exhausting the supply of von Willebrand factor.[5] This does not tend to cause spontaneous bleeding and, tranexamic acid can be used to prevent bleeding during minor procedures. In contrast, major surgery will require platelet counts to be normalized and a normal ristocetin cofactor activity should be confirmed before proceeding.[5] Cytoreductive therapy (Tables 9a and 9b) is also recommended for patients who are resistant or intolerant to phlebotomy.[7] While a definition for resistance

Table 9a. Conventional medical intervention for Polycythemia vera

Drug	Mode of action	Advantages	Limitations
Hydroxyurea	• Cytoreductive agent • Ribonucleotide reductase inhibitor that reduces intracellular deoxynucleotide triphosphate pools, thus inhibiting DNA synthesis resulting in cytotoxicity.	• Reduces thrombotic risk relative to phlebotomy alone	• Optimal dose must be determined through individual titration. • Associated with secondary cancers including squamous cell and basal cell carcinoma and breast cancer due to inhibition of TP53. • Myelosuppressive • Side effects include ulceration, skin lesions and gastrointestinal toxicities. • Not safe in pregnancy due to teratogenic properties • May not be well tolerated in the long term. • Does not cause molecular remission
Anagrelide	• Suppression of transcription factors required for proliferation of megakaryocytes.	• Effective platelet reduction • Non-Leukemogenic	• The most common side effects include: Bloating or swelling of the face, arms, hands, lower legs, or feet; body aches or pain; burning, itching, numbness; chest pain; congestion, cough, difficult or labored breathing, dryness, or soreness of the throat; heart palpitations; fever; rapid weight gain; lymphadenopathy

Table 9b: Conventional medical intervention for Polycythemia vera (continued)

Drug	Mode of action	Advantages	Limitations
Pegylated Interferon α 2a	• Cytoreductive agent • Immune regulation via binding to interferon α receptors 1 and 2	• Effective at reducing the neutrophil JAK2V167F allele burden. • Complete molecular remission can be achieved. • Effective cytoreduction. • Reduces symptoms of pruritus. • Reduced splenomegaly, but not back to normal size • Non Leukemogenic • Less toxic than Ruxolitinib • Safe in pregnancy	• Not always effective at reducing erythrocytosis as it can activate erythroid gene expression. • Immunosuppressive • Thyroid and liver toxicity • Side effects include flu like symptoms, atrial fibrillation/arrhythmias, neuropathy and depression. • Hypothyroidism • Autoimmune and endocrine disorders may occur in a minority of patients • Stimulates hematopoiesis so phlebotomy may remain necessary.
Ruxolitinib	• JAK1/2 inhibitor	• Effective and durable reduction of symptoms • Reduced splenomegaly relative to hydroxyurea • Phlebotomy is often no longer necessary • Molecular remission rates similar to interferon. • Normalisation of severe iron deficiency. • Reduces risk of thrombosis • Effective cytoreduction. • May increase the effectiveness and tolerability of pegylated interferon α -2b (phase II trials) • Non-myelotoxic • Non-Leukemogenic	• Immunosuppressive • Thrombocytopenia • Anemia • Increased rates of non-melanoma skin cancers

to phlebotomy has not been standardized, some have recommended criteria of more than three venesections a year, as this has been linked to an increased risk of thrombosis, and/or severe symptomatic iron deficiency.[5] Criteria for intolerance includes fainting episodes or a blood phobia. Iron supplementation may be cautiously recommended for patients suffering from severe symptoms of iron deficiency due to phlebotomy, with the resulting increase in hematocrit being treated with cytoreductive therapies.[17]

HU is the most common first line treatment for high risk patients.[16] However, some clinicians avoid the use of HU in those under 40 years of age, particularly women, in favor of interferon (Tables 9a and 9b).[1] Where HU is used in women of childbearing age, it is recommended that patients receive appropriate contraception and that treatment with HU is stopped three months before any intended conception.[9] The use of HU for the treatment of PV is not strictly evidence based, as much of the evidence has been extrapolated from the treatment of ET. However there have been small studies that have demonstrated a benefit in terms of a reduction in thrombotic risk.[7]

Resistance or intolerance to HU will develop in a quarter of patients.[16,17] Resistance is associated with a poor prognosis and may manifest as continued pruritus, progressive or symptomatic splenomegaly or a requirement for an increased frequency of phlebotomy and/or uncontrolled myeloproliferation.[8,9,18] Intolerance to HU usually manifests as mucosal or cutaneous lesions or genital and leg ulcers. For older patients, actinic keratosis or non-melanoma skin cancers may occur. It is recommended that patients on this treatment avoid sun exposure.[9,16] Links between HU and secondary leukemia remain unproven and are

unsupported by long term follow up studies.[7,9] In contrast the use of alkylating agents and radiotherapy are associated with increased rates of leukemia.[7]

Ruxolitinib has been approved as a second line drug for patients who become resistant or intolerant to HU or interferon therapy.[19,20] There is an estimated response duration of three years.[18,20] However, the immunesuppressive properties have been shown to cause an increased rate of infection, with a 4.6% 10-year mortality risk rate in PV patients.[18] Ruxolitinib affects both the innate and adaptive immune system, impacting natural killer cells, dendritic cells, and regulatory T lymphocytes in terms of both activation and proliferation. While the risk of infection is lower in PV than for the other MPNs, (according to epidemiological studies based on data from Swedish registries) an in-depth risk assessment is recommended for infection prior to commencing treatment.[18] Patients should be screened for hepatitis B and those who test negative should be offered vaccination. Antiviral prophylaxis should be considered for those who test positive. Herpes zoster and pneumococcal vaccination should also be considered.[18] Additional risk factors would include tuberculosis or travel to tuberculosis endemic areas. It is also important to educate patients on the signs of infection and encourage them to seek early medical attention.

New therapies continue to be added to the arsenal. Additional therapies, currently under investigation include Ropeginterferon α 2b, which has a longer half-life than the 2a form and appears to be better tolerated.[21] It was approved for use as a monotherapy in patients without splenomegaly by the European Medicines Agency in 2019.[22] In addition, histone deacetylase (HDAC) inhibitors such as Givinostat and murine double minute 2 (MDM2) inhibitors such as Idasanutlin have also entered clinical trials.[8] Histone deacetylase catalyzes the removal of acetyl groups from lysine residues on histones, leading to down regulation of tumor suppressor genes. Inhibitors are being investigated for use as anticancer drugs. Givinostat has been shown to suppress clonogenic activity of JAK2V617F positive cell lines in *in vitro* models. It has demonstrated a hematological and molecular response in phase II trials and appears to be well tolerated.[22] MDM2 has been shown to be over expressed in JAK2617F positive cells, leading to a reduced the tumor repressor protein, p53, response and thus DNA damage. Idasanutlin is a selective small molecule MDM2 antagonist, shown to reactivate p53, stimulating apoptosis of JAK2617F CD34 (hematopoietic marker) positive cells.[23] It is also in phase II trials and appears to be well tolerated.[6,23]

Conclusion

Although PV is a relatively rare disorder, the discovery of the JAK2V167F mutation has revolutionised the diagnosis and prognostic evaluation of the disease, making it the subject of intense research. The underlying mechanisms for fibrotic and leukemic transformation of PV are not yet fully understood. They are likely to be multifactorial, but improved knowledge and application of DNA analysis may be beneficial for stratification of patients for risks of both transformation and thrombosis.[5] As technological advances increase the identification of genes associated with transformation, future research may yield new targets for therapy that may prevent transformation, thus improving the prognosis for patients with this disease.[1] Further research is also needed to investigate how newer therapies can be integrated into existing treatment plans for optimal results, as well as their impact on disease progression, allowing targeted and individualised treatment for patients.[4,5,18]

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