REVIEW ARTICLE

From Driver Mutations to Genomic Classification: Current & Future Perspectives on Myeloproliferative Neoplasms

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ABSTRACT

Myeloproliferative neoplasms (MPNs) encompass a heterogeneous group of chronic, clonal haematopoietic stem cell neoplasms that harbor the propensity to undergo leukaemic transformation. Epidemiological data on MPNs especially pertaining to non-Caucasian populations is limited, and the molecular pathogenesis of MPN remains unclear. Although the discovery of MPN driver mutations in *JAK2, MPL* and *CALR* in the last decade has revolutionised disease management, the mutations are not specific for any MPN subtype. The management of MPNs is further challenged by substantial genetic and phenotypic heterogeneity that exist between and within MPN subtypes as well as other myeloid diseases. In this review, we focus on the classical Philadelphia chromosome (Ph)-negative MPNs – polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF); providing an overview on the current understanding of the disease at a clinical and molecular standpoint while discussing the present challenges and future opportunities in the management of MPNs.

Keywords: Myeloproliferative neoplasm, Mutation, Molecular pathogenesis, Disease management

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INTRODUCTION

First described by William Dameshek (1) in 1951 as 'somewhat variable manifestations of proliferative activity of the bone marrow cells' (p.374), myeloproliferative neoplasms (MPNs) are a heterogenous group of chronic, clonal haematopoietic disorders with a shared characteristic of cellular hyperproliferation. These disorders arise from genetic mutations that lead to the constitutive activation of signalling pathways responsible for haematopoiesis, and the subsequent overexpansion of specific myeloid compartments. Current classification guidelines divide MPNs into several subtypes, but phenotypic overlaps within and between MPN subtypes as well as other myeloid disorders are common. Due to the chronic nature of MPNs, patients are often asymptomatic upon diagnosis. However, the benign myeloproliferation masks the ability of the disease to transform into acute leukaemia which is often fatal. Despite decades of research, understanding of MPN pathogenesis remains limited. Notably, chronic myeloid leukaemia (CML) is the only MPN with a distinct molecular marker – the Philadelphia (Ph) chromosome [t(9;22)] encoding *BCR-ABL1*. Targeted monotherapy with tyrosine kinase inhibitors has greatly improved survival rates in CML (2). However, such success has not been duplicated for the remaining MPNs (also known as Ph-negative MPNs). In the last decade, breakthrough discoveries of driver mutations in *JAK2, MPL* and *CALR* in Ph-negative MPNs have been made. Nevertheless, no mutation discovered thus far is specific for any Ph-negative MPN subtype, and therapy is largely aimed at disease control rather than cure. This review captures the current clinical and molecular landscape of MPN and provides perspectives on future opportunities to improve the management of the disease.

POPULATION-BASED STUDIES: LIMITED UNDERSTANDING OF THE GLOBAL DISEASE BURDEN

Global epidemiological studies on MPNs remain scarce. A 2014 meta-analysis of 28 studies (3) estimated the pooled annual incidence rates of ET, PV and PMF at 1.03, 0.84 and 0.47 cases per 100,000 population (95% CI:0.70–1.01, 0.58–1.80 and 0.34–0.65) respectively. Peak incidence is around 50 to 70 years of age (ET=50-

60 years, PV=60 years, and PMF 60-70 years), with a slight female predominance in ET, a slight male predominance in PV, and an almost equal incidence in men and women in PMF (2). However, there is great variability in reported incidence rates of ET, PV and PMF across different studies, ranging from 0.01–2.8, 0.2–2.3 and 0.5–1.5 cases per 100,000 population respectively (2). This variability may be due to: 1) differences in etiology between populations, i.e. environmental, lifestyle or familial/ethnic factors, 2) changes in the MPN diagnostic criteria since the discovery of the *JAK2* V617F driver mutation, 3) under-reporting of disease cases due to the chronic and sometimes asymptomatic nature of MPNs, and 4) variation in the management of MPN patients and registries between nations (3-6). Only a handful of nations have published data on MPN population incidence since the 2014 meta-analysis, namely the United States, Canada, Norway, Sweden and South Korea (7-11). Populations in Asia and Africa are the most understudied. Available studies report on frequency of single or multiple driver mutations in patients with MPN, but not population incidence (12-18). Due to the limited data, the true global incidence of MPN, especially pertaining to non-Caucasian populations, remains unknown.

HETEROGENEITY IN SYMPTOMS: DIVERGENCE IN CLINICAL OUTCOME

Presenting features of MPN include constitutional symptoms such as headache, fatigue, pruritus, facial plethora and weight loss, as well as hepatosplenomegaly which occurs due to the sequestration of excessive blood cells and/or proliferation of abnormal haematopoietic progenitor cells (19). In ET and PV, common causes of morbidity are thrombosis and ischemia due to an increased red cell mass and high platelet numbers that lead to the blockage of vessels. Individuals with ET may also experience haemorrhage of mucosal surfaces (particularly in the gastrointestinal and respiratory tracts) due to platelet dysfunction and acquired von Willebrand disease. In PMF, about 90% and 50% of patients are affected by splenomegaly and hepatomegaly respectively, and more than 50% of PMF patients suffer from constitutional symptoms (20, 21). However, many individuals with MPNs are asymptomatic upon diagnosis. The actual proportion of asymptomatic PV cases has not been reported, but more than 50% of ET cases and as many as 30% of PMF cases have been discovered incidentally through routine blood and/or physical examinations (2).

Primary causes of MPN morbidity and mortality are thromboembolic and haemorrhagic complications as well as disease progression (i.e. into myelofibrosis (MF)) or transformation into acute myeloid leukaemia (AML) (Fig. 1A) (22). In general, PMF is associated with the poorest disease prognosis and outcome, followed by PV, and ET is the most indolent of all MPNs (23). For

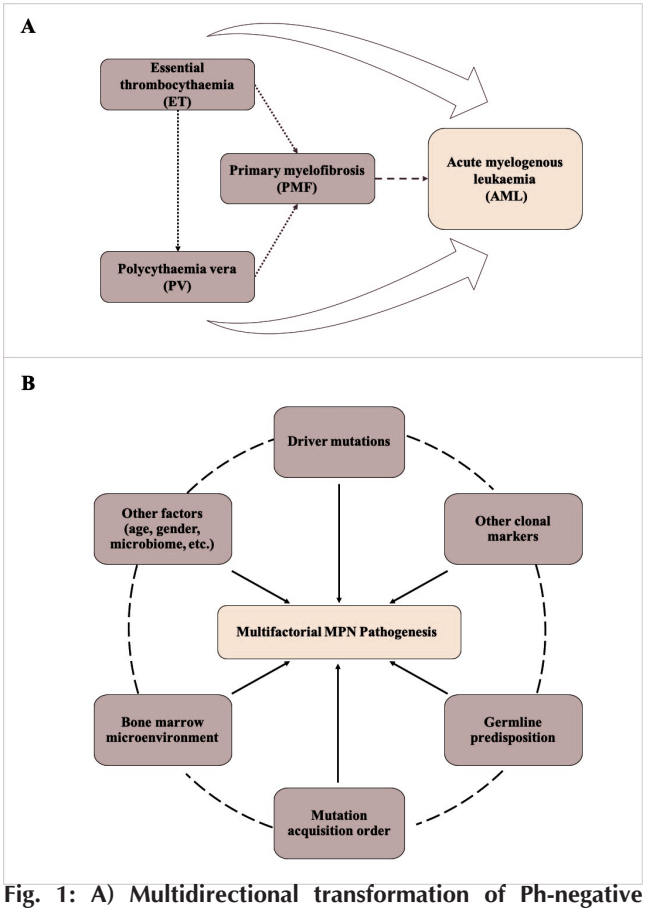

Fig. 1: A) Multidirectional transformation of Ph-negative MPNs and B) Multifactorial pathogenesis of MPN. (A) Each MPN subtype has the potential to result in bone marrow failure by progressing to PMF or even AML (2). Adapted from Nangalia et al., 2016 (27). (B) Several factors have been implicated in the pathogenesis of MPN. These factors are not necessarily exclusive and may exist in combination.

PMF, the median survival upon diagnosis is 3 to 5 years (24). Around 20% of patients succumb to leukaemic progression, and comorbidities such as cardiovascular events, infection and bleeding are also common causes of death (25). For PV, the 10-year projected survival rate is more than 75%, whereas less than 5% and 10% of cases undergo leukaemic transformation and fibrotic progression respectively (26). For ET, survival rates are similar to that of the general population - the 15-year survival is around 80% (which can worsen thereafter due to thrombosis or haemorrhage), whereas the 10-year risk of fibrotic or leukaemic transformation is less than 1% (23, 27, 28).

MULTIFACTORIAL PATHOGENESIS: GENETICS, CELLULAR, MICROENVIRONMENT & OTHER FACTORS

The first evidence that MPNs originate from the clonal expansion of haematopoietic stem cells (HSCs) upon acquiring mutations that confer a selective growth advantage that drive the myeloproliferative phenotype was recorded in 1976 (30). HSCs are self-renewing, multipotent cells located in the bone marrow that can

give rise to all the blood cell lineages. Traditionally, the process of haematopoiesis is modelled as a hierarchy that begins with HSCs and is followed by intermediate progenitor cells that differentiate into fully specialised myeloid and lymphoid cells. In MPNs, studies have detected the presence of driver mutations in HSCs and all mature cell lineages (31-33); supporting the concept that MPN pathogenesis originates from cells at the apex of the haematopoietic hierarchy (29). However, none of the driver mutations identified are MPN subtype-specific, nor does the absence of driver mutations exclude disease. Each MPN subtype also has the potential to progress in a stepwise fashion; culminating in bone marrow failure due to MF, ineffective haematopoiesis or even transformation into AML (Fig. 1A) (2). As such, there is ongoing debate that MPNs should not be treated as separate distinct entities, but as a single continuum, whereby disease outcome is determined by various factors (Fig. 1B). We discuss the different factors in the following sections:

i) Driver mutations

Research in the last decade has led to the discovery of MPN driver mutations in three genes: *JAK2*, *MPL* and *CALR* which are found in more than 95% of PV, ET and PMF cases. Similar to the *BCR-ABL1* oncogene associated with CML, these mutations lead to the constitutive activation of cytoplasmic/receptor tyrosine kinases in signalling pathways critical for myelopoiesis, such as the signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK), and phosphatidylinositol-3′-kinase (PI3K) pathways (Fig. 2). In 2005, the first and commonest MPN driver mutation, *JAK2* V617F was discovered (4, 34-36). Found in 95% of PV cases and 50-60% of ET and PMF cases, *JAK2* V617F occurs in exon 14 of *JAK2* and results in the constitutive activation of the *JAK2* tyrosine kinase and JAK/STAT signalling; a pathway critical for myelopoiesis (Fig. 2). Other *JAK2* MPN driver mutations were subsequently discovered in *JAK2* V617F-negative PV in the form of insertions and deletions in *JAK2* exon 12 (Fig. 3A) (37, 38). Studies on MPN patients and mouse models (37, 39, 40) observed that these *JAK2* exon 12 mutations resulted in an isolated erythrocytosis phenotype, and therefore greater amplification of downstream signalling effects as compared to the *JAK2* V617F mutation.

In 2006, two research groups identified JAK/STAT activating mutations in the thrombopoietin (TPO) receptor gene, *MPL* (Fig. 2) (42, 43). Mutations in *MPL* are usually substitutions (i.e. *MPL* W515L and W515K) that occur in exon 10 and induce constitutive activation of *MPL* (42-45). A less common mutation, *MPL* S505N results in an activated conformation of the protein (46-48). Following the discovery of driver mutations in *JAK2* and *MPL*, other elements of JAK/STAT signalling have become the centre of research focus. However, these efforts have not resulted in further significant findings. Nevertheless, two individual whole-exome sequencing

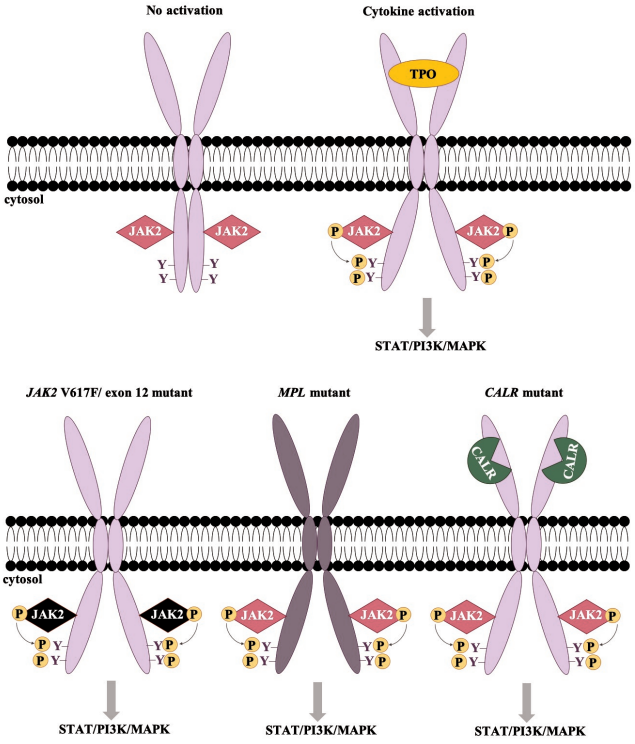

Fig. 2: Activation of the thrombopoietin (TPO) receptor *MPL*. (Top) JAK2 binds with the intracellular domain of the inactive MPL receptor homodimer. Binding of TPO to the extracellular domain of the *MPL* receptor homodimer induces an "active" conformational change that enables transphosphorylation of the attached *JAK2* molecules. The activated *JAK2* molecules then phosphorylate tyrosine residues on the intracellular domain of the MPL receptor homodimer, which then activate downstream signalling pathways such as signal transducer and activator of transcription (STAT), phosphatidylinositol-3′-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. (Bottom) The *JAK2* V617F and exon 12 mutations as well as mutations in *MPL* lead to ligand-independent activation of downstream signalling pathways. Similarly, mutant *CALR* associates with *MPL* to result in ligand-independent activation of downstream signalling pathways. Information sourced from Nangalia et al., 2017 (39).

studies in 2013 (49, 50) discovered an unlikely candidate in the form of calreticulin (*CALR*), which is a chaperone protein with no direct role in cell signalling, cell fate or even haematopoiesis. All reported *CALR* mutations are frameshifts that are caused by insertions or deletions in exon 9; which results in a novel C-terminus of the protein. Subsequent studies suggest that mutant *CALR* interacts with *MPL* to cause constitutive JAK/STAT signalling; resulting in MPN phenotypes similar to those of *MPL* mutations (Fig. 2) (51-55).

Of note, the thrombopoietin receptor *MPL* is selectively expressed on HSCs and cells of the megakaryocytic lineage (56). Constitutive activation of *MPL* results in increased megakaryopoiesis and a thrombocytosis phenotype, in keeping with the observation that *MPL* and *CALR* driver mutations occur almost exclusively in ET and PMF, but not in PV (Fig. 3A) (57). In contrast,

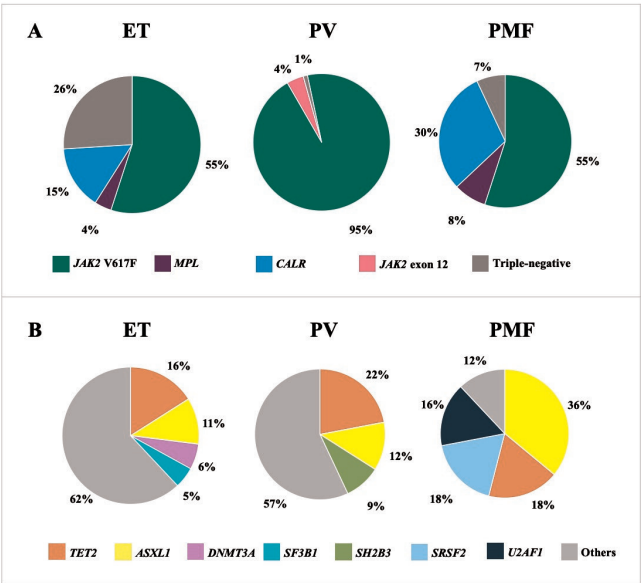

Fig. 3: Relative frequencies of (A) *JAK2, MPL* and *CALR* driver mutations and (B) other clonal marker mutations in ET, PV and PMF. (A) The most prevalent driver mutation in MPNs is the *JAK2* V617F mutation, followed by *CALR* and *MPL* mutations. The latter two gene mutations are found primarily in ET and PMF. A proportion of MPN cases are TN. (B) Mutations in other clonal marker genes are not only identified in TN MPNs, but are also found in the presence of driver mutations. Data compiled from Tefferi et al., 2017 and Grinfeld et al., 2017 (54, 55). TN, Triple-negative.

the *JAK2* tyrosine kinase is expressed ubiquitously on cells of all lineages. Although mutations in *JAK2* exon 12 are rare and reported only in PV (37, 38), the *JAK2* V1617F mutation has been observed in a spectrum of MPN phenotypes, ranging from asymptomatic PV to severe MF (4, 34-36).

Evidence suggests that variations in *JAK2* gene dosage, or more specifically, *JAK2* V617F allelic burden affects the MPN phenotype. Individuals with PV are reported to have the highest *JAK2* V617F allelic burden, followed by individuals with PMF, whereas individuals with ET have the lowest *JAK2* V617F allelic burden (22). A study on MPN patient-derived induced pluripotent stem cells found that only *JAK2* V617F heterozygous cells produced thrombopoietin-independent megakaryocyte colonies, suggesting that *JAK2* V617F heterozygosity drives thrombopoiesis, whereas *JAK2* V617F homozygosity drives erythropoiesis (59). In *JAK2* V617F-positive ET and PV, *JAK2* V617F homozygosity is associated with higher haemoglobin levels and leukocyte counts, incidence of aquagenic pruritus and splenomegaly, as well as risk of thrombosis and progression to MF (60-62). Similarly, studies on transgenic mouse models found that an increase in *JAK2* V617F expression resulted in a shift in MPN phenotype from ET to PV (63, 64), and that *JAK2* V617F-homozygosity resulted in accelerated MF (65).

ii) Other clonal markers

Despite the discovery of MPN driver mutations in *JAK2*, *MPL* and *CALR*, around 10% of patients are "triple-negative (TN)"; i.e. they do not harbour mutations in any of these three genes (Fig. 3A) (48, 57). Clonal markers which are generally found across myeloid diseases are not only present in TN MPN, but can also be present with MPN driver mutations (Fig. 3B) (48, 66, 67). These markers include genes involved in: 1) cell signalling pathways, i.e. JAK/STAT pathway elements (e.g. *SH2B3, CBL, KIT, GNAS, GNB1*) and RAS pathway elements (e.g. *KRAS, NRAS, NF1, PTPN11*); 2) epigenetic regulation i.e. *TET2, IDH1 and IDH2, DNMT3A, EZH2, ASXL1, CUX1, MLL3* and *PHF6*; 3) regulation of the cell cycle and/or apoptosis i.e. *BCOR, PPM1D, RB1, STAG2* and *TP53*; 4) regulation of gene transcription i.e. *GATA2, NFE2*, and *RUNX1*; and 5) mRNA processing i.e. *U2AF1, ZRSR2, SF3B1*, and *SRSF2*. Although the roles of such clonal markers in MPN pathogenesis are less understood, each clonal marker is correlated with changes in the MPN phenotype. For example, mutations in mRNA processing elements and epigenetic regulators are associated with an MF phenotype, leukaemic transformation and poor survival in MPNs (29, 68, 69). As such, clonal markers are important prognostic factors and have since been incorporated as additional diagnostic criterion for MPNs (2, 41, 58, 70).

iii) Germline predisposition

Genetic variants linked to an increased MPN predisposition have also been identified. According to Nangalia et al. (41), such variants can be classified into two groups: 1) common variants in populations that mildly increase MPN predisposition, and 2) rare variants in pedigrees that demonstrate higher penetrance of specific alleles. Common variants include the *JAK2* 46/1 (GGCC) haplotype as well as single nucleotide polymorphisms (SNPs) present in or close to *TERT* (rs2736100) and *MECOM* (rs2201862), which in combination are estimated to account for 55% of population attributable risk (71-74). Rare variants include mutations in *RBBP6* (found to affect the p53 apoptotic pathway and increase the risk of developing further mutations) (75), as well as a duplication of 14q32.2 (associated with the overexpression of *ATG2B* and *GSKIP*) and the SNP rs9376092 in the intergenic region between *HBS1L* and *MYB* (*HBS1L-MYB*), both of which promote megakaryopoiesis and the development of an ET phenotype (73, 76). Other novel variants in *JAK2* and *MPL* have also been identified in TN ET and MF patients, most of which were germline than somatic (48, 67). MPN predisposition loci in *TERT, SH2B3, GF11B, ATM, CHEK2*, and *TET2* have also been reported (77).

iv) Order of mutation acquisition

As MPNs are clonally heterogenous, differences in the order of mutation acquisition and clonal architecture can contribute to differences in MPN phenotype and prognosis between individuals. Studies on *JAK2*-mutated MPN patients with *TET2* (78) and *DNMT3A*

(79) mutations found that those who are *JAK2*-first have larger homozygous *JAK2*-mutated subclones as well as 'double-mutant' subclones (with *TET2* or *DNMT3A* mutations) and often present with PV, whereas patients who are *TET2*-first or *DNMT3A*-first have a dominant 'single mutant' subclone (with mutated *TET2* or *DNMT3A* only) and often present with ET. Nangalia et al. (41) proposed three mechanisms that may drive the differences between different patterns of mutation acquisition, i.e., the first mutation may alter 1) the response of a HSC to the second mutation, 2) HSC differentiation, resulting in altered progeny populations in which the second mutation can arise, and 3) the number and function of mature progeny, therefore affecting the bone marrow environment.

v) Haematopoietic stem cells (HSCs)

HSCs are traditionally understood as a relatively homogenous population of cells with equal regenerative capacity and multipotency. However, tremendous molecular and functional heterogeneity has recently been revealed within the HSC population (80). HSC transplant experiments in mice (81-84) found that only a small proportion of HSCs were 'balanced' and produced a roughly equivalent multi-lineage output, whereas the majority of HSCs were 'biased' towards the production of certain lineages (80). To date, lymphoid-, myeloid- and platelet-biased HSCs have been characterised (81-90). Although direct evidence is lacking, Mead et al. (91) hypothesised that HSC lineage bias may contribute to MPN phenotypic heterogeneity by facilitating more direct pathways of haematopoiesis, i.e. an MPN driver mutation which occurs in a platelet-biased HSC may promote an ET phenotype, whereas the same driver mutation which occurs in a myeloid-biased HSC may promote a PV phenotype. A 2014 study observed the expansion of myeloid-restricted progenitor cells originating from single malignant HSCs that were reconstituted in transgenic mice, providing further supporting evidence to support the lineage-bias hypothesis (92).

vi) Bone marrow microenvironment

The behaviour of HSCs is not only determined by their intrinsic properties, but also by extrinsic factors in the bone marrow microenvironment. Bone marrow components, such as endothelial cells, osteoblasts, osteoclasts, and stromal cells secrete signalling molecules that are essential for the regulation of normal HSC function, but also play an essential role in MPN pathogenesis. Several *in vivo* studies (93-96) have provided evidence that the disruption of the bone marrow niche can result in an MPN phenotype. Bone marrow stromal cytokines have been associated with the proliferation of malignant MPN clones or the inhibition of the growth of normal clones (97-103). Moreover, expansion of the malignant clone also releases cytokines that create a malignant 'self-reinforcing' niche that favors the survival and proliferation of malignant MPN HSCs than normal HSC (104). The aberrant production of cytokines by malignant HSCs has been found to cause neural damage, fibrosis, and increased microvessel density in the bone marrow (105-108). In addition, studies reporting the development of human PMF features in mice MF-xenografts, i.e. constitutive cellular mobilization into the peripheral blood, splenomegaly, and leukaemic transformation suggest that normal HSCs can be affected by cytokine signals from malignant HSCs (109, 110). Interestingly, there is considerable overlap between the cytokine profiles of PV, ET and PMF, thus supporting the idea that the diseases belong to the same biological spectrum (111, 112).

vii) Other factors

Aging is the primary risk factor for MPN development. Mutations that lead to clonal haematopoiesis of indeterminate potential (CHIP) in genes such as *ASXL1, DNMT3A, SF3B1, SRSF2* and *TET2* are rare in individuals below the age of 40 but increase exponentially in elderly individuals (41, 113). Studies on HSCs found that aged HSCs have reduced lymphoid potential that results in a myeloid-bias, as well as decreased self-renewal and marrow-homing ability as compared to young HSCs (114-117). Gene expression profiling of aged HSCs revealed an overall increase in transcription with a loss of transcriptional regulation; the systemic down-regulation of genes involved in mediating lymphoid potential, chromatin remodelling and the preservation of genomic integrity; as well as the up-regulation of genes involved in mediating myeloid potential, leukaemic transformation, stress response, inflammation, and protein aggregation (114, 115, 118).

Aside from age, other factors such as gender or even the microbiome has been associated with changes in MPN phenotype. PV is reported to be more common in males, whereas ET is more common in females (2). Although *JAK2* V617F homozygosity is reported to occur equally in both genders, it is associated with PV in males but ET in females (61). Therefore, it is possible that male androgens affect the expansion of homozygous *JAK2* V617F subclones that result in a phenotypic skew towards PV, whereas female estrogens combined with iron deficiency (which impedes erythropoiesis and promotes thrombopoiesis) in pre-menopausal women result in a phenotypic skew towards ET (29). Other erythropoiesis-constraining conditions such as thalassemia or low erythropoietin levels may also lead to ET rather than PV (119). Moreover, studies on the role of the microbiota in haematological diseases suggest that the disruption in the normal gut microbiota is associated with clinically significant outcomes; whereby some pathogens are associated with disease initiation (120).

THE EVOLUTION OF MPN DISEASE CLASSIFICATION

The classification of MPNs has evolved with each new discovery. According to the World Health

Organization (WHO) (2), accurate definition of disease entities requires an integrated, multimodal approach involving the evaluation of clinical features, morphology, immunophenotype, cytogenetics, as well as molecular genetics. However, prior to the discovery of MPN driver mutations, MPN (then known as chronic myeloproliferative disorders) classification by the WHO was based on then available research by the Polycythaemia Vera Study Group and was heavily reliant on morphological analysis (121, 122). Several important revisions have since been made to the WHO MPN classification guidelines. The latest 2016 revision to the WHO classification include: 1) testing for the presence of driver mutations or other clonal markers as diagnostic criterion for PV, ET or PMF, 2) decreasing of the platelet count and hemoglobin level threshold for the diagnosis of ET and PV respectively, 3) clear definition of minor diagnostic criteria that may impact the accurate diagnosis and prognosis of prefibrotic PMF, and last but not least, 4) standardization of morphologic criteria and emphasis on histologic diagnosis, i.e. the requirement for a bone marrow biopsy for the classification of any MPN subtype (2, 28, 123-125). Like any disease, accurate diagnosis of an MPN subtype is critical for predicting disease prognosis and determining the choice of therapy for the best outcome.

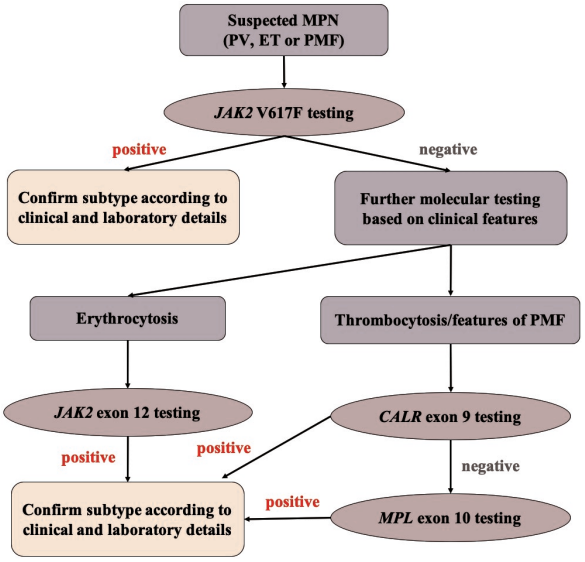

Fig. 4: Example of a clinical molecular diagnostic workflow for MPNs that incorporates molecular tests for driver mutations. Due to the relatively high frequency of *JAK2* V617F mutations in MPNs, *JAK2* V617F testing is the primary step in MPN confirmatory diagnostics. Current practices do not necessitate further molecular tests upon a positive *JAK2* V617F result (27).

DISEASE MANAGEMENT: PROGRESS MADE BUT MUCH TO BE DESIRED

Based on the latest WHO classification guidelines, a typical diagnostic workflow incorporates molecular testing for MPN driver mutations and a bone marrow aspirate and/or trephine biopsy which is correlated with the results of a full blood count (Fig. 4). In order to exclude CML, molecular testing for *BCR-ABL1* is usually conducted as a preliminary test. Erythropoietin assays and lactase dehydrogenase (LDH) assays may also be required to confirm an MPN diagnosis, whereas other tests may be required to disassociate comorbidities from the presenting symptoms such as anaemia and thrombocytosis. Clinical therapeutic decisions may be then guided using prognostic models such as the International Prognostic Scoring System (IPSS) and the Dynamic IPSS (DIPSS) which group patients into categories (i.e. high-, intermediate-, and low-risk groups) based on factors such as age, blood cell count, driver mutations, and previous history of thrombosis (126, 127).

However, substantial heterogeneity in phenotype and genotype between and within MPN categories, and even other myeloid disorders pose a significant challenge towards MPN management. Certain mutations may be more prevalent in one subtype as compared to the other (e.g. *JAK2* V617F occurs in >90% of PV cases as compared to 50-60% of ET and PMF cases), but none are exclusive to any single MPN subtype. Mutations in *JAK2, CALR* or *MPL* can also be present in other haematological malignancies such as myelodysplastic syndromes (MDSs), myelodysplastic/myeloproliferative neoplasms (MDS/MPNs), and acute myeloid leukaemia (AML) (2, 125). Clinical features of MPNs such as bone marrow fibrosis or haematocrit levels are continuous variables that are fundamentally challenging to assign to individual MPN entities (128). Morphological features used for diagnosis, such as the degree of bone marrow fibrosis, are based on subjective interpretation and prone to high inter-observer variability (129-131). One multicentre study that compared the grading of reticulin fibrosis between local pathologists and an expert panel found that the agreement rate was only 56% (132). Hence, the management of MPNs may be improved with a novel classification method such as one that is based on genomics, that does not rely on continuous variables for assigning disease categories.

Therapeutic decisions are made based on the disease burden, prognosis and mutational landscape for each individual patient throughout their course of disease. For patients with ET or PV, the risk of thrombosis, haemorrhage, and evolution to PMF and AML (and less commonly MDS) is relatively high. In comparison, almost all patients with PMF develop anaemia and have a higher incidence of splenomegaly, greater symptom burden and poorer survival (133). To date, allogeneic stem cell transplantation remains the only curative option for MPNs (91). However, the procedure is usually not considered due to age-related co-morbidities and high transplant-related mortality (134). Hence, the goal of MPN therapy is to reduce symptom burden, risk of thrombohaemorrhagic complications, as well as the risk

of disease progression and malignant transformation. Treatment options include phlebotomy, hydroxyurea (HU), aspirin, anagrelide, pegylated interferons that target HSCs, and JAK inhibitors such as ruxolitinib (135, 136).

Ruxolitinib became the first and only JAK inhibitor to be approved for MPN therapy, specifically for intermediate/high-risk PMF and HU-resistant/intolerant PV after the COMFORT I and II trials as well as the RESPONSE trial (137-140). The benefits of ruxolitinib therapy such as marked symptom reduction has spurred efforts to investigate the use of ruxolitinib in ET and high-risk PV (141, 142). However, ruxolitinib therapy has limited ability in inducing complete molecular remission and regression of bone marrow fibrosis (143-145). Moreover, the inhibition of *JAK2* results in on-target anaemia and thrombocytopenia, which hinders dose-optimisation of ruxolitinib especially for PMF patients with severe thrombocytopenia (146). As such, a variety of novel agents are still being investigated for use in MPN therapy, whether alone or in combination with already-approved therapeutic agents such as ruxolitinib. These include various agents such as sotatercept (an anti-anaemia agent), anti-fibrotic agents, apoptosis-inducing agents, hypomethylating agents and BCL-2-homology domain 3 (BH3)-mimetics, along with inhibitors of human double minute 2 (HDM2), histone deacetylase (HDAC), telomerase, cyclin-dependent kinase (CDK), the PI3K pathway, and Hedgehog signalling, as well as other JAK inhibitors (146). However, none are close to regulatory approval and many studies have been discontinued due to toxicity concerns (142, 146).

PRECISION MEDICINE: ROLE OF GENOMIC CLASSIFICATION AND PREDICTION MODELS

As previously described, MPN disease management is challenged by the heterogeneity within and between MPN subtypes. Nevertheless, this challenge can be overcome by shifting away from a classification scheme that is dependent on clinical and morphological observation, to one that is based on genomics (147). In a recently developed prognostic model (127), 2035 patients with ET, PV and PMF were stratified into eight genomic groups: 1) MPN with TP53 disruption or aneuploidy, 2) MPN with chromatin or spliceosome mutation, 3) MPN with *CALR* mutation, 4) MPN with *MPL* mutation, 5) MPN with homozygous *JAK2* or *NFE2* mutation, 6) MPN with heterozygous *JAK2* mutation, 7) myeloproliferation with other driver mutation, and 8) myeloproliferation with no known driver mutation. Compared to current prognostic schemas such as the IPSS, DIPSS, and International Prognostic Score for ET (IPSET), the novel prognostic model showed superiority in performance and revealed substantial heterogeneity in the disease outcomes within current prognostic categories, especially within categories of "intermediate-risk" (127). However, the implementation of such a genomic prognostic model into the global clinical setting will require further validation studies, as well as the widespread adoption of comprehensive genetic profiling such as next-generation sequencing (NGS) technology in clinics worldwide.

A variety of myeloid NGS panels are currently available commercially – most are amplicon-based for short turnaround time and target anywhere from 20 to 50 myeloid neoplasm-associated genes with important prognostic information (148, 149). However, several challenges accompany the implementation of NGS into routine clinical diagnostics of myeloid malignancies. The first challenge is the ability to differentiate leukaemia-associated mutations from polymorphisms, passenger mutations and CHIP; requiring robust bioinformatics tools and large population datasets (148, 150). Next are the technical challenges associated with the NGS platform. Frequently, it is challenging to discriminate true genetic alterations from artefacts that may arise throughout the NGS workflow (151-153). Although Sanger sequencing is usually employed to validate NGS sequences, the Sanger technique has lower sensitivity (detection limit of around 15 to 20%) as compared to NGS (detection limit as low as 1%), making it unsuitable for the detection of low allele fractions (154). The setting up of the NGS platform in a clinical laboratory is also associated with high instrument cost and requires an interdisciplinary approach that involves constant interaction between clinicians, laboratory scientists and technicians that are well-experienced in NGS (148). Although the price of NGS panels will continue to decrease, the cost of purchasing and maintaining sequencing equipment remains high. The NGS workflow itself, which encompasses the initial panel design to the analysis and interpretation of results, is also technically tedious and time-consuming. Nonetheless, clinical laboratories currently employ traditional molecular techniques in parallel with NGS in order to provide results that will inform patient therapy at the earliest possible time point.

CONCLUSION

Although substantial progress has been made in the field of MPNs, there remains gaps that hamper our complete understanding of MPNs. Ongoing efforts to investigate the role of various factors in MPN pathogenesis will hopefully take us closer to this reality. In this era of genomics and targeted therapy, genetic screenings of patients with myeloid malignancies such as MPNs are becoming increasingly accessible and routine. The success of the JAK inhibitor ruxolitinib in the treatment of MPN patients provides hope that other agents can be equally developed as targeted therapies for individuals who test positive for other molecular markers. As such, a classification scheme that is based on genomics has the potential to better inform management decisions. Nevertheless, there is a severe lack of data especially

for populations outside of Europe and North America. More studies are required in order to reduce population-specific bias and improve the understanding of the disease burden of MPNs in non-Caucasian populations. In addition, better experimental models, both *in vivo* or *in vitro* should be developed to investigate the effect of single or a combination of various MPN-associated mutations on disease progression, and to overcome the challenges of genotypic and phenotypic heterogeneity in the management of MPN. Lastly, the setting-up of molecular diagnostic laboratories and mainstreaming of genetic screening practices, such as the adoption of NGS technology in clinics worldwide will enable the provision of state-of-the-art diagnostics and prognostics to patients in a cheap, fast and accessible manner, and at the same time improve patient outcomes in the long run.

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