Myeloproliferative neoplasia: a review of clinical criteria and treatment

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ABSTRACT

Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) belong to the group of Philadelphia chromosome-negative myeloproliferative neoplasia (Ph- MPN). MPNs are clonal bone marrow stem cell disorders characterised by a proliferation of one or more of the myeloid, erythroid or megakaryocytic cell lines. Due to the different affected cell lines, MPNs show typical clinical and histological features. In 2005, a mutation in the JAK2 gene was discovered which generated more insight into the pathogenetic working mechanism of MPNs. However, the treatment of MPN patients is still mainly only palliative, although progress in reducing the symptoms of MPN patients has been made. This review will give a general overview of MPN patients for internal medicine physicians.

KEYWORDS

MPN, myeloproliferative neoplasia, essential thrombo-cythemia, polycythemia vera, primary myelofibrosis, treatment myeloproliferative neoplasia

HAEMATOPOIESIS

Haematopoiesis is the development of the cellular components of the blood. The formation and development of blood cells is initiated by the haematopoietic stem cells (HSCs). HSCs are primitive cells capable of self-renewal and differentiation. Due to the self-renewal capability, at least one of the daughter cells possesses the same HSC characteristics as the mother cell after cell division. During the entire life of an individual, the stem cell pool is maintained due to the self-renewal capability of the HSCs and supplies cells for multilineage haematopoiesis. [1,2]
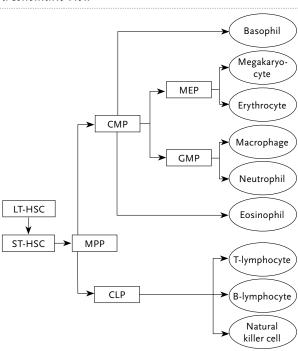
Currently it is considered that long-term repopulating HSCs (LT-HSC) differentiate into a short-term repopulating HSC (ST-HSC) and, as schematically shown in *figure 1*, they will differentiate further into multipotent progenitor cells (MPP) only capable of differentiating into the myeloid lineage or the lymphoid lineage. The common myeloid progenitors (CMP) give rise to megakaryocyte-erythroid progenitors (MEP), which differentiate into megakaryocytes and erythrocytes, and granulocyte-monocyte progenitors (GMP), which differentiate into macrophages and neutrophil granulocytes. The eosinophilic and basophilic granulocytes differentiate directly from the CMP. The common lymphoid progenitors (CLP) differentiate into T- and B-lymphoid cells and natural killer cells (*figure 1*). The progeny that arises from HSCs progressively loses its self-renewal capacity and gradually becomes more restricted to one lineage. [3,4]

HSCs require intrinsic and extrinsic factors for their activities provided by the stem cell niche. The interaction of HSCs with the stem cell niche determines whether the HSCs remain in a quiescent state or proliferate to progenitor cells and differentiate into mature blood cells. [5,6]

MYELOPROLIFERATIVE NEOPLASIA

Myeloproliferative neoplasia (MPNs) are clonal bone marrow stem cell disorders involving a multipotent haematopoietic stem cell, characterised by proliferation of one or more lineages of the myeloid, erythroid and megakaryocytic cell lines. This proliferation results in increased numbers of granulocytes, erythrocytes or platelets in the peripheral blood respectively. [7] William Dameshek was the first to introduce the term 'myeloproliferative disorders' in 1951 including essential

Figure 1. *Development of haematopoietic stem cells, a schematic view*

HSC = haematopoietic stem cells; LT-HSC = long-term repopulating HSC; ST-HSC = short-term repopulating HSC; MPP = multipotent progenitor; CMP = common myeloid progenitor; MEP = megakaryocyte-erythroid progenitor; GMP = granulocyte-macrophage progenitor; CLP = common lymphoid progenitor.

thrombocythemia (ET), polycythemia vera (PV), primary myelofibrosis (PMF), chronic myelogenous leukaemia (CML) and erythroleukaemia (Di Guglielmo syndrome). These disorders were grouped together based on their similarities in clinical phenotype and the belief that there was an underlying undiscovered stimulus responsible for the proliferative activity of bone marrow cells in these myeloproliferative disorders.[8]

According to the World Health Organization (WHO) 2008 criteria, MPNs are now divided in classical MPNs which carry the Philadelphia (Ph+) chromosome (chronic myeloid leukaemia) and classical MPNs which do not carry the Philadelphia (Ph-) chromosome, including ET, PV and PMF. The Philadelphia chromosome is a result of t(9:22) with the *BCR-ABL1* fusion gene.[9] In this article the classical Ph- MPNs are highlighted.
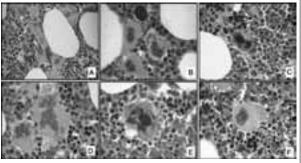
Clinical and histological criteria of MPN

The typical features of ET are thrombotic and haemorrhagic complications, although most patients are asymptomatic. Transient ischaemic attacks, erythromelalgia and Budd-Chiari syndrome are complications which can occur in ET patients or can develop before the diagnosis of ET is apparent. Bleeding complications are a result of an extremely high platelet count resulting in an acquired von Willebrand disease; von Willebrand factor will be proteolysed with increasing platelet counts.[10] Histomorphological findings in the bone marrow of ET patients are loose clusters of predominant large to giant megakaryocytes. The megakaryocytes exhibit a normal maturation with hyperlobulated and staghorn-like nuclei (*figure 2*). No marked left-shifting of the erythroid or myeloid cell line is apparent. The presence of reticulin is extremely rare in ET patients at presentation and very few patients (<10%) develop myelofibrosis during their disease course, known as post-ET myelofibrosis. ET patients have a risk of approximately 2% to develop acute myeloid leukaemia (AML).[11,12]

Polycythemia vera is characterised by a trilineage proliferation of the erythroid, myeloid and megakaryocytic cell line, usually resulting in mainly increased erythrocytes and often also leucocytes and blood platelets. Patients also display a persistently raised haemoglobin and haematocrit level. The clinical features of PV patients are vascular occlusive events, enlarged spleen, aquagenic pruritus (intense itching after a hot bath or shower) and haemorrhagic complications after injuries and surgery. In about 30% of the patients PV will develop to myelofibrosis, known as post-PV myelofibrosis, and leukaemic transformation will occur in about 10% of the PV patients.[12] The bone marrow of PV patients displays panmyelosis and therefore an increase in cellularity. The megakaryocytes reveal a range from small to giant megakaryocytes without maturation defects of nuclei and cytoplasm and are arranged in loose clusters (*figure 2*). There is always a proliferation and often a left-shifting of the myeloid cell lineage and especially of the erythroid precursor cells. Slightly increased reticulin fibrosis can be seen in the bone marrow.[11]

Figure 2. *Examples of morphological features in megakaryocytes*

A. Dense clustering (HE, 630x) B. Loose clustering (HE, 1000x) C. Dysmorphic nucleus (HE, 1000x) D. Hyperlobulated nucleus (HE, 1000x) E. Staghorn nucleus (HE, 1000x) F. Cloud-like nucleus (HE, 1000x)

In primary myelofibrosis the patient's complaints and symptoms depend mainly on the degree of anaemia and splenomegaly. The typical early symptoms are fatigue, weight loss, night sweating and fever. These constitutional symptoms are believed to be mediated by the abnormal release of cytokines from clonal megakaryocytes as a result of emperipolesis. When the fibrosis is in an advanced stage, the complaints are, apart from the constitutional symptoms, paleness due to anaemia, hepatosplenomegaly, spleen infarct and osteosclerosis. Budd-Chiari syndrome can be a feature of early-phase disease and can be the presenting symptom.[12,13] In the bone marrow of prefibrotic PMF an overall hypercellularity is evident including prominent growth of abnormally differentiated and giant megakaryocytes. The megakaryocytes reveal hypolobulated, cloud-like and hyperchromatic nuclei and demonstrate dense clustering (*figure 2*), often accompanied by left-shifted granulocyte proliferation. In the prefibrotic PMF reticulin fibrosis may be absent, but during the disease course reticulin fibrosis increases, finally resulting in collagen fibrosis with osteosclerosis. Leukaemic transformation occurs in about 10% of the PMF patients.[14] However, the symptoms listed above are not strictly limited to ET or PV or PMF patients, in fact they can occur in all three classical Ph- MPN, such as bleeding complications (spontaneous or after surgery), thrombosis and fatigue. MPN patients may even be asymptomatic in the early phases of the disease and it may be a coincidence that an MPN disease is discovered by abnormal blood counts or by diseases which are features of early-phase MPN, such as Budd-Chiari syndrome, heart attack, cerebral vascular accident, pulmonary thrombus and deep venous thrombosis. An important factor in thromboembolic events is the $JAK2^{V617F}$ mutation. No differences in thromboembolic events were seen between heterozygous and homozygous $JAK2^{V617F}$ PV patients, in contrast to homozygous ET patients, who show increased risk of cardiovascular events compared with heterozygous and wild-type ET patients. It was also shown that ET and PV patients with a higher allele burden have a higher risk of thrombotic events.[15] This indicates an important risk factor for the $JAK2^{V617F}$ mutation in the development of thrombosis. The $JAK2^{V617F}$ occurrence rate in patients with thrombosis of the deep veins (DVT) and pulmonary embolism (PE) is low, therefore a general $JAK2^{V617F}$ screening is not recommended among patients with spontaneous DVT and PE. This is in contrast to patients who present with splanchnic and intrahepatic vein thrombosis; these patients show a high prevalence of the $JAK2^{V617F}$ mutation and a diagnosis of ET or PV should be kept in mind.[16,17]
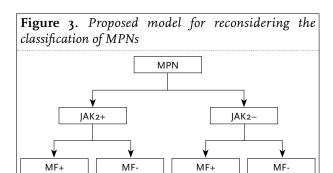
The Polycythemia Vera Study Group (PVSG) made the first attempt to establish diagnostic criteria for the Ph-MPNs in 1967. The diagnostic criteria were updated several times during the following decades and are even now widely used by haematologists. However, the appropriate use of bone marrow biopsy (BMB) histology as a diagnostic tool was neglected. To stress the relevance of a BMB, the WHO added a set of histological diagnostic criteria in 2001. The recent discovery of the $JAK2^{V617F}$ mutation and the recognition of pre-fibrotic PMF resulted in the 2008 WHO classification of MPNs.[18-20] However, the early phases of ET, PV and PMF are difficult to distinguish on morphology alone as they share many morphological characteristics. It was shown by Wilkins *et al.* that some of the histological criteria as described in the WHO classification were difficult to reproduce.[21] Nevertheless, it is very important to distinguish these three MPN subtypes reliably in the early phase, because of a different risk of thromboembolic complications of PV and the worse survival rate of PMF patients compared with ET patients, who have a normal life expectancy.[21,22]

Although Ph- MPNs are divided into three clinically distinct entities, the use of three distinct diagnoses can also be questioned; ET, PV and PMF show a great abundance of overlap in their morphological characteristics, clinical signs and symptoms and can also share the same molecular mutation ($JAK2^{V617F}$). A proposed simplistic model for revision of the MPN classification is shown in *figure 3*. It might be more reasonable to divide the MPNs into JAK2 positive and negative diseases and subdivide them into patients with and without myelofibrosis.[23]

THE JAK2 MUTATION AND MPN

In 2005, several groups identified a mutation in the tyrosine kinase domain of JAK2 in MPN patients, resulting in a substitution of valine for phenylalanine at position 617 of JAK2 ($JAK2^{V617F}$). The first genetic step is an acquired point mutation and results in a heterozygous

Figure 3. *Proposed model for reconsidering the classification of MPNs*

MPN = myeloproliferative neoplasia; JAK2+ = positive for the JAK2V617F mutation; JAK2- = wild-type JAK2; MF+ = myelofibrosis present in the bone marrow; MF- = myelofibrosis absent in the bone marrow.

mutational status. The homozygous $JAK2^{V617F}$ mutation is the result of mitotic recombination between homologous chromosomes 9p and results in loss of heterogeneity of 9p (LOH) and is a second genetic step in the aetiology of the MPNs.[24-28] The $JAK2^{V617F}$ mutation is present in granulocytes, erythroblasts and myeloblasts and in all erythropoietin (EPO)-independent erythroid colonies. The erythroid colonies with the $JAK2^{V617F}$ mutation are able to grow in the absence of EPO. Therefore, the $JAK2^{V617F}$ mutation also results in factor-independent growth of various haematopoietic cell lines.[29] Further, the receptors of bone marrow progenitor cells are hypersensitive to thrombopoietin (TPO, stimulates proliferation and differentiation of megakaryocytes), EPO (stimulates erythroblasts), stem cell factor (SCF, induces proliferation and self-renewal of multipotent haematopoietic progenitors) and granulocyte-stimulating factor (GSF, stimulates proliferation and differentiation of granulocytes). The hypersensitivity for these cytokines results in specific stimulation of the megakaryopoiesis, erythropoiesis and granulopoiesis.[30]

The $JAK2^{V617F}$ mutation is present in >95% of the PV patients and in approximately 50% of the ET and PMF patients.[15,31] The $JAK2^{V617F}$ mutation deregulates the JAK2 kinase activity. The mutation is located in the JH2 domain of the JAK2 gene, which negatively regulates the activity of the kinase domain, JH1. Valine 617 and cysteine 618 both maintain the kinase domain of JAK2 in an inactive state. Substitution of valine 617 for phenylalanine destabilises this inhibitory interaction, resulting in increased JAK2 kinase activity. Altogether, this suggests that there is a sustained JAK2 activation, while the feedback mechanism has been destroyed with a growth factor independent activation.[24] PV patients without the $JAK2^{V617F}$ mutation virtually all have a $JAK2^{V617F}$ exon 12 mutation.

Also, more early genetic abnormalities are currently being defined and related with disease development.

TREATMENT OF MPN

The current treatment of MPN patients is mostly supportive, while standard therapy has not been defined firmly. The treatment of ET and PV patients should be done according to their risk stratification for the occurrence of thromboembolic processes (*table 1* and *table 2*) as evaluated in a large prospective study of the European Collaboration on Low-dose Aspirin in Polycythemia (ECLAP).[32] Age greater than 60 years and a previous history of thrombosis were found to be risk factors for thrombosis in both ET and PV. Is one of these two criteria present the ET and PV patient is at high risk, whereas if none of the criteria are present ET and PV patients are at low risk. ET and PV patients who have platelets >1000 x

Table 1. *Risk stratification of patients with ET and PV for the occurrence of thrombosis*

Risk category	Age >60 years or history of thrombosis	Generic cardiovascular risk factors
Low	No/No	No
Intermediate	Platelets >1000 x 10^9/l	Yes
High	Yes/No or No/Yes	Irrelevant

Table 2. *Treatment of ET and PV according to their risk stratification*

Risk category	ET	PV
Low	Low-dose aspirin* if microvascular disturbances are present	Phlebotomy + low dose aspirin*
Intermediate	Low-dose aspirin* if microvascular disturbances are present	Phlebotomy + low dose aspirin*
High	Low-dose aspirin* if microvascular disturbances are present + hydroxyurea^	Phlebotomy + low dose aspirin* + hydroxyurea^

***In the case of major bleeding or presence of von Willebrand syndrome, aspirin is a contraindication; ^hydroxyurea intolerance or resistance, use anagrelide or peg-INF-α.**

10^9/l are of intermediate risk to develop thrombosis or if they have any of the following risk factors: hypertension, hypercholesterolaemia, smoking and diabetes mellitus (*table 1*). These are generic cardiovascular risk factors, and their role is still controversial. Other possible risk factors, which have to be validated in prospective studies, might be leukocytosis and the presence of the $JAK2^{V617F}$ mutation, although the latter is controversial.

ET patients belonging to the low-risk or intermediate-risk category and without any symptoms do not need therapy; however, aspirin is recommended to prevent microvascular disturbances as erythromelalgia, although major bleeding or presence of von Willebrand syndrome are contraindications for the use of aspirin. High-risk ET is an indication for the use of hydroxyurea (HU), which inhibits thrombocyte, erythrocyte and leucocyte production, combined with low-dose aspirin if thrombosis or microvascular symptoms are present, of course in the absence of contraindications (*table 2*).[23,32-35] In the MRC-PT-1 trial researchers compared HU plus aspirin with anagrelide plus aspirin in ET patients at high risk for thrombosis, observing that HU plus low-dose aspirin is superior to anagrelide plus low-dose of aspirin.[36]

The administration of aspirin to PV patients has been widely investigated. In 1986, the PVSG concluded that aspirin was ineffective and dangerous, due to increased gastrointestinal bleeding and intracerebral haemorrhage, based on a randomised trial of 163 PV patients receiving either 900 mg/day aspirin plus dipyridamole or radioactive

phosphorus (^{32}P).[37] However, more studies on the administration of aspirin have been done, resulting in the conclusion of the safe use of a considerably lower dose of aspirin in PV patients. The Gruppo Italiano Studio Policitemia Vera demonstrated the safe use of low-dose aspirin (40 mg/day) in PV patients.[38] The study by Landolfi et al.[39] showed a significant reduction in the combined risk of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, pulmonary embolism or major venous thrombosis with 100 mg/day of aspirin. Therefore, low-dose aspirin plus phlebotomies are recommended in the low-risk and intermediate-risk category.[23]

In 1953, the most effective treatment of PV included phlebotomies combined with radioactive phosphorus (^{32}P) resulting in prolonged survival; however ^{32}P was shown to be leukemogenic.[40] The PVSG study group conducted a randomised trial comparing phlebotomy alone with ^{32}P plus phlebotomy and with chlorambucil plus phlebotomy. Patients treated with phlebotomy alone showed a higher incidence of thrombosis in the first three years of treatment. After three to five years of study, a considerable number of patients treated with ^{32}P or chlorambucil developed acute leukaemia, lymphoma and carcinomas of the gastrointestinal tract and skin, compared with those treated with phlebotomy alone. Therefore, patients treated with phlebotomy alone had a better overall median survival of 13.9 years than patients treated with chlorambucil (8.9 years) or ^{32}P (11.8 years).[33] The PVSG also compared HU with phlebotomy; a slightly higher incidence of acute leukaemia, less myelofibrosis and fewer deaths among the patients treated with HU were apparent.[41]

Interferon-α is able to inhibit *in vitro* proliferation of haematopoietic progenitors and inhibition of the thrombopoietin-induced MPL receptor signalling resulting in megakaryopoiesis repression. The use of IFN-α in PV patients was shown to be effective and non-leukemogenic. However, the use of IFN-α has been limited due to its toxicity, parenteral administration and costs.[42,43] The development of pegylated (peg) forms of IFN resulted in improved tolerance, efficacy and fewer side effects.[44,45] Peg-IFN-α has been demonstrated to have clinical advantages, high rates of molecular response and lower toxicity in phase II trials in PV as well as ET patients.[46,47]

PV patients belonging to the low-risk or intermediate-risk category with high haematocrit level are treated with phlebotomies in order to obtain normal haematocrit levels (<0.45 l/l) plus low-dose aspirin, if no contraindications are present. If PV patients show poor compliance to phlebotomy or if they show progressive myeloproliferation, cytoreductive therapy should be given. The high-risk group should be treated with myelosuppression, with HU as the drug of choice (*table 2*). Anagrelide or peg-INF-α is used in PV and ET patients in case of intolerance or resistance to HU, to control platelet count or in those who develop side effects to HU; however, long-term efficacy and safety features are still unknown.[23,35,48]

The prognosis of PMF patients is worse than that of ET or PV patients (median survival six *vs* 20 years) and the disease course is not significantly modified by drug therapy, therefore treatment of PMF is mainly palliative. However, there is a wide heterogeneity in presentation and evolution among PMF patients. Therefore, the International Prognostic Scoring System (IPSS) uses five risk factors for estimating the survival of PMF patients at the time of diagnosis: age >65 years, constitutional symptoms (weight loss, fever, excessive sweating), haemoglobin level <10g/dl, leucocyte count >25 x 10^9/l and circulating blasts >1%. Based on this system PMF patients can be categorised in the low-risk group (0 risk factors present), intermediate-1 (1 risk factor present), intermediate-2 (2 risk factors present) and high-risk group (≥3 risk factors present).[49] IPSS has been modified to Dynamic IPSS (DIPSS) with the same five risk factors to estimate survival during the disease course, while acquisition of additional risk factors modifies patients outcome.[50] Recently, the DIPSS was upgraded to DIPSS-plus by incorporating three independent prognostic factors, including the need for red cell transfusion, thrombocytopenia <100 x 10^9/l and unfavourable karyotype (including +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3) or 11q23 rearrangement). Based on the DIPSS-plus PMF patients are categorised in the low (no risk factors), intermediate-1 (1 risk factor), intermediate-2 (2 or 3 risk factors) and high (≥4 risk factors) risk group. Unfavourable karyotype and thrombocytopenia both predict leukaemic transformation in PMF patients. If the patient needs red cell transfusion, the patient belongs to the intermediate-risk group, while the patient displays two risk factors: anaemia and red cell transfusion need.[51]

A wait-and-see approach is justified in PMF patients belonging to the low- or intermediate-1 risk group, while the median survival of these patients exceeds 15 and six years respectively.[51] This relatively long median survival does not justify the risks of an allogeneic stem cell transplantation (alloSCT) or the start of investigational drug therapy. There is also no evidence to support the use of conventional drug therapy in low- or intermediate-1 risk group patients if the patients do not have complaints which can be treated (*table 3*).[13] However, if PMF patients suffer from splenomegaly, the first drug of choice is HU and in the worst case splenectomy is indicated. Indications for splenectomy include symptomatic portal hypertension, drug-refractory splenomegaly with severe symptoms, transfusion-dependent anaemia, marked thrombocytopenia and uncontrollable haemolysis due to severe complications that can occur. Irradiation therapy of the spleen transiently reduces spleen size and reduces

Table 3. *Treatment of PMF according to their risk stratification*

Risk category	PMF
Low	Wait-and-see or conventional drug therapy
Intermediate-1	Wait-and-see or conventional drug therapy
Intermediate-2	Hydroxyurea* or experimental drugs or alloSCT
High	Hydroxyurea* or experimental drugs or alloSCT

*Hydroxyurea intolerance or resistance, use peg-INF-α.

the incidence of pancytopenia. Patients usually experience relief of constitutional symptoms when splenomegaly is treated. In the case of non-hepatosplenic extramedullary haematopoiesis (located mainly in the thoracic vertebral column or in lymph nodes, lung pleura, small bowel, peritoneum, urogenital tract and heart) low-dose irradiation therapy is indicated.[52,53]

In patients belonging to the intermediate-1 risk group who suffer from the risk factor they display, conventional drug therapy should be given; anaemia can be treated with androgens, danazol, corticosteroids, thalidomide or lenalidomide. Thalidomide plus prednisone and lenalidomide plus prednisone show higher response rates with decreased toxicity. Thalidomide and lenalidomide are also effective in PMF patients with unfavourable karyotype. A recent study by Holle *et al.*[54] showed an improvement in haemoglobin and thrombocyte counts and a reduction in spleen size and bone marrow fibrosis in patients with PMF, post-ET and post-PV myelofibrosis treated with thalidomide. However, side effects are toxicity and mainly neurotoxicity. More promising might be lenalidomide, which shows fewer side effects with similar improvement in haematopoiesis.[53] The use of erythropoiesis-stimulating agents in myelofibrosis is not recommended due to the risk of splenomegaly exacerbation.[52,55]

PMF patients in the intermediate-2 and high-risk group have an indication for therapy, as well as regular therapy as investigational drug therapy, due to the low survival rates in these patients (*table 3*). In the presence of thrombocytosis, leukocytosis, splenomegaly or bone pain, there is an indication for hydroxyurea. Anaemia can be treated as indicated for the intermediate-1 risk group and splenectomy is also indicated as stated above.[23,53,56-60]

The only potentially curative treatment in PMF patients is allogeneic stem cell transplantation with an overall three-year survival ranging from 30 to 60%. AlloSCT can induce graft versus host disease (GvHD), which can be divided into acute GvHD and chronic GvHD, with an incidence of about 30 to 43% and 30 to 48%, respectively.[61-63] However, despite the high rate of death and the high risk of chronic morbidity due to GvHD, alloSCT is justified in PMF patients belonging to the intermediate-2 or high-risk group, while the median survival of these patients is three years and one year[64] respectively (*table 3*). The three-year overall survival of PMF patients after alloSCT ranges from 37 to 58%.

Future treatment

New therapeutic strategies include JAK inhibitors and imatinib mesylate. Imatinib mesylate (tyrosine kinase inhibitor) is used in the treatment of chronic myelogenous leukaemia and has been shown to reduce spleen size and to reduce the proliferative activity in PV patients.[65] Several JAK inhibitors have been developed since the discovery of the $JAK2^{V617F}$ mutation in 2005, among them ruxolitinib (INCB018424), SAR302503 (TG101348), CYT387, lestaurtinib (CEP701) and SB1518.

Ruxolitinib is a JAK1 and JAK2 inhibitor which was tested in a phase I/II trial. Patients showed responses after one to two months including reduction of spleen size and improvement of constitutional symptoms including fatigue, weight loss, night sweats and pruritus. A more than 50% decrease in total symptom score after 24 weeks occurred in 46% of the patients compared with 5% for the placebo group. Haematological side effects were anaemia and thrombocytopenia (grade 3 or 4). Non-haematological toxic effects were low grade and infrequent. After 60 days the overall survival of the patients treated with ruxolitinib was higher compared with the placebo group (hazard ratio = 0.67). Allele burden was minimally decreased and ruxolitinib was shown to be effective in patients with the $JAK2^{V617F}$ mutation, but also in patients without the JAK2 mutation.[66,67] Ruxolitinib is now being tested in a phase III trial.

In a recent study by Tefferi *et al.* 51 patients were enrolled in the phase I/II COMFORT trial experiencing a very rapid relief of symptoms related to the presence of myelofibrosis and splenomegaly. However, the occurrence of serious anaemia and thrombocytopenia, loss or lack of response, disease progression, patient/physician choice often associated with lack of response, and death during the study prompted 47 patients to discontinue with ruxolitinib treatment. During treatment discontinuation, acute relapse of symptoms and splenomegaly were experienced by most patients, which sometimes required hospitalisation. This observation stresses the need for careful disclosure of the ruxolitinib withdrawal syndrome to myelofibrosis patients. Further, treatment discontinuation should be done under close supervision in a gradual tapering schedule, although the tapering schedule does not guarantee that the withdrawal symptoms will not occur.[68] However, these side effects and the occurrence of ruxolitinib withdrawal syndrome do not counteract the benefits MPN patients with myelofibrosis experience with ruxolitinib treatment. SAR302503 is a selective JAK2 inhibitor inducing rapid spleen size reduction and improvement of constitutional

symptoms. Further, the majority of patients with leukocytosis and thrombocytosis at baseline achieved normal blood counts. A significant decrease in the $JAK2^{V617F}$ allele burden was observed. Grade 1 self-limiting side effects were nausea, diarrhoea and vomiting. Haematological side effects of grade 3 to 4 were anaemia, thrombocytopenia and less frequently neutropenia.[69] SAR302503 is being tested in a phase II trial at the moment.

CYT387 inhibits the JAK1 and JAK2 gene. First results are promising; improvement in spleen size, anaemia and constitutional symptoms. Side effects were headache and thrombocytopenia.[70] CYT387 is currently under investigation in a phase I/II trial.

Lestaurtinib inhibits JAK2 and JAK3 and improves spleen size, transfusion dependency and cytopenias. No effect was seen on the $JAK2^{V617F}$ allele burden. Side effects were diarrhoea, anaemia and thrombocytopenia.[71] Currently, lestaurtinib is under investigation in a phase II trial.

SB1518 is a highly selective JAK2 inhibitor and was well tolerated in a phase I trial with a decrease in spleen size and improvement in clinical symptoms.[72] SB1518 is currently being tested in a phase I/II trial.

Another promising drug might be pomalidomide, a second-generation immunomodulatory drug. Pomalidomide was shown to improve anaemia (in 25% of patients treated with 0.5 mg/day and in 36% of patients treated with 3.0 mg/day) and platelet count in patients with ≤100 x 10^9/l (in 58% patients treated with 0.5 mg/day).[73,74]

Hypomethylating agents have also been investigated. The most promising is decitabine, which was tested in a phase II study in 21 MPN patients with myelofibrosis, showing a reduction of 61% in circulating CD34$^+$ cells. ITF2357, a histone deacetylase inhibitor, was shown to resolve pruritus in most patients, to reduce splenomegaly in 38% of the patients and showed a trend in reducing the $JAK2^{V617F}$ allele burden.[75]

Everolimus (RAD001) inhibits the mammalian target of rapamycine (mTor) and was shown to reduce spleen size, to complete resolution of systemic symptoms and to reduce anaemia. Side effects were worsening of anaemia in 30% of the patients and grade two neutropenia or thrombocytopenia, although infrequent.[76]

The JAK inhibitors are the most promising new drug strategies for MPN patients with improvement in quality of life and relatively minimal side effects. However, the long-term safety of these agents and whether they prolong survival should be determined. Therefore JAK inhibitors should only be started as a form of therapy in myelofibrosis patients belonging to the intermediate-2 or high-risk group.

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