Diagnosis and treatment of myelofibrosis: a personal perspective

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Practice Points

- Achieving an accurate diagnosis is critically important and cannot be achieved on trephine biopsy only; integration of clinical, molecular, blood film and trephine reports is required.
- The International Prognostic Scoring System (IPSS), dynamic IPSS or dynamic IPSS plus score are useful but not yet validated for postpolycythemia vera and postessential thrombocythemia myelofibrosis (MF). Molecular-based scores are being developed.
- MF remains an incurable disease. Current treatments are aimed at individual disease features such as anemia. Allotransplant should be considered early and offered to patients with aggressive disease. Interferon continues to be of interest in early-phase disease, but is of limited utility.
- JAK inhibitors are now being widely trialed in patients with MF. The first-in-class agent ruxolitinib has proven to show a benefit in spleen and symptom control, and current data support the fact that it will prolong survival.
- A host of other JAK inhibitors are being developed and some appear to have differential benefits. For example, reduction in marrow fibrosis and less myelosuppression for SAR302503, and potenial anemia response for CYT387. Phase III studies with such agents are underway.
- Different experimental strategies for the management of MF that are currently being tested include everolimus (RAD001), panobinostat and other histone deacetylase inhibitors, telomerase inhibitiors and the use of combination therapies.

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SUMMARY Myelofibrosis can arise *de novo* or following one of the other Philadelphia-negative myeloproliferative neoplasms. The differential diagnosis may be challenging and can include other entities which may also express the *JAK2* V617F mutation, such as chronic myelomonocytic leukemia and/or refractory anemia with ring sideroblasts. Traditionally a difficult disease to treat with only a small proportion of patients eligible for a curative bone marrow transplant, this field has recently changed radically with the introduction of JAK inihibitors, the first in class being ruxolitinib.

The first reported case of myelofibrosis (MF) was probably described by Hueck in 1879 as a 'peculiar leukemia'; he described two cases with splenomegaly, fibrous material in the bones and constitutional symptoms. MF is now described as a clonal proliferative disorder of the hematopoietic stem cells, unconnected with the *BCR-ABL* translocation, and clinically characterized by bone marrow fibrosis, splenomegaly, leukoerythroblastosis, extramedullary hematopoiesis and a constellation of debilitating symptoms. MF encompasses primary MF (PMF) and secondary forms, which include postpolycythemia vera (PPV) and postessential thrombocythemia (PET) MF [1]. This field has seen a rapid pace of change since the original descriptions of a V617F mutation in *JAK2* in approximately 50% of patients with MF [2–5].

This has led to the recognition of JAK1 and JAK2 activation as a consistent finding and the description of several other mutations that may activate JAK2 directly or indirectly, or affect the epigenetic processes within the hematopoietic stem cell. More dramatic have been clinical reports with JAK inhibitors and their striking benefits for patients with this disorder.

Here we provide details of the approach we use to manage this disease, incorporating these new data as well as a discussion of recent data and future directions for therapy.

Achieving an accurate diagnosis

The diagnosis of PMF, as defined by WHO, is based on a combination of clinical, morphological, cytogenetic and molecular features [6]. Furthermore, the diagnoses of PPV- and PET-MF have recently been clarified by the International Working Group for Myelofibrosis Research and Treatment, with the criteria being adopted by WHO. It is also important to recognize that fibrotic change in the marrow may occur due to other causes, some of which, including myelodysplasia, chronic myeloid leukemia and chronic myelomonocytic leukemia may be difficult to distinguish from MF **(Figure 1)**. The *JAK2* V617F mutation may be present in many of these conditions since it is not specific for a myeloproliferative neoplasm. Furthermore, even when utilizing the WHO criteria, specific diagnostic difficulties may arise in differentiating between essential thrombocythemia (ET) and some early forms of PMF [7,8]. In view of these limitations, we utilize the diagnostic criteria proposed by Campbell and Green for PMF **(Table 1)** [9], as well as for PPV- and PET-MF **(Table 2)**, these were recently recommended via a formal guideline process [10]. We also exercise great caution in making a diagnosis of pre-fibrotic MF unless clear minor criteria to support this are present.

We believe that careful evaluation of all of these criteria is critical to achieve an accurate diagnosis; in our practice this is done in the context of a multidisciplinary meeting synthesizing all available morphological, molecular and cytogenetic data with the clinical history. We also consider that the diagnosis should be formally reviewed if, during its course, the disease is displaying atypical characteristics or changes in character. The reason for this practice is that the *JAK2* V617F clone is thought to display genetic instability [11], although this is controversial and this, perhaps combined with the mutagenic properties of some therapies, means that different clones may arise. This may underlie, for example, the finding of a *BCR/ABL*-positive clone during the course of the disease.

Clinical features & prognosis

PMF affects 0.5–1.5 per 100,000 of the population and most people are diagnosed in the sixth decade of life, with the median age of MF diagnosis 67 years, and there is roughly equal involvement of the sexes. Exact data concerning

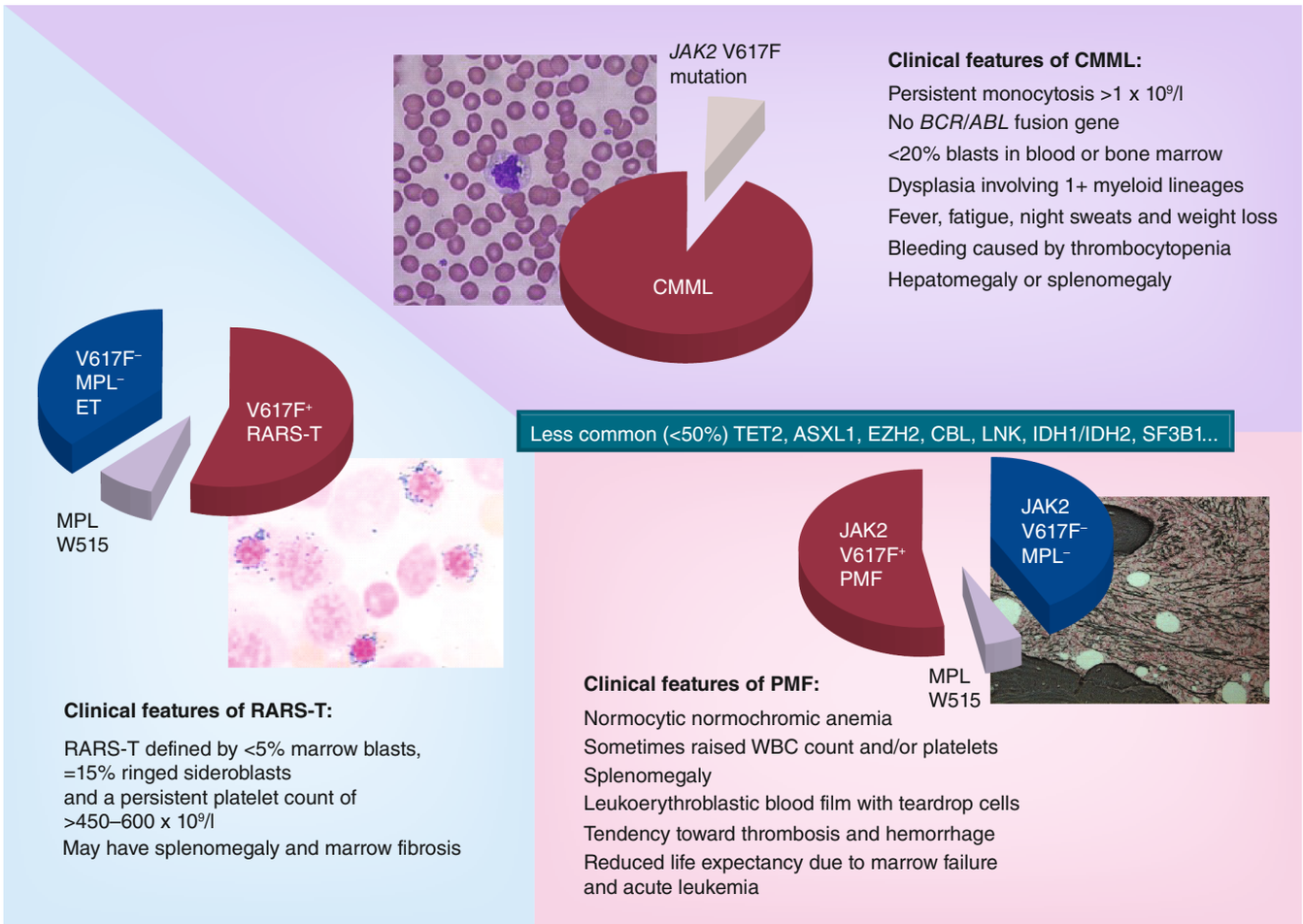

Figure 1. Clinical features of myelofibrosis and diseases, such as chronic myelomonocytic leukemia and refractory anemia with ring sideroblasts, which share common features and may be confused clinically.
CMML: Chronic myelomonocytic leukemia; ET: Essential thrombocythemia; MPL: Murine proliferative leukemia; PMF: Primary myelofibrosis; RARS-T: Refractory anemia with ring sideroblasts thrombocytosis; WBC: White blood cell.

the prevalence of PET-MF and PPV-MF are actually not known. PET-MF and PPV-MF arise at a variable and quite unpredictable rate in patients with ET or PV, and although several factors have been proposed including, for example, the use of venesection in PV rather than cytoreductive therapy, we have a very poor understanding of the factors involved in the transformation from ET or PV to PET-MF or PPV-MF in the process and even less of how to moderate them.

The clinical features of MF are, in general, common to PMF, PET-MF or PPV-MF and include progressive anemia, leukopenia or leukocytosis, thrombocytopenia or thrombocytosis and multiorgan extramedullary hemopoiesis, the latter most commonly causing hepatomegaly and symptomatic splenomegaly, portal hypertension and a spectrum of symptoms that increase in prevalence and severity with advanced disease. Early death frequently

Table 1. British Committee for Standards in Haematology diagnostic criteria for primary myelofibrosis.

A1	Bone marrow fibrosis at least grade 3 (on 0–4 scale).
A2	Pathogenetic mutation (e.g., in JAK2 or MPL), or absence of both BCR-ABL1 and reactive causes of bone marrow fibrosis
B1	Palpable splenomegaly
B2	Unexplained anemia
B3	Leukoerythroblastosis
B4	Teardrop red cells
B5	Constitutional symptoms†
B6	Histological evidence of extramedullary hematopoiesis

Diagnosis requires A1 + A2 and any two B criteria.
†Only drenching night sweats, weight loss >10% over 6 months, unexplained fever (>37.5°C) or diffuse bone pains (no other symptoms are included).
MPL: Murine proliferative leukemia.

Table 2. Diagnostic criteria used in our practice for postpolycythemia vera or postessential thrombocythemia myelofibrosis.

A1	Bone marrow fibrosis ≥3 (on 0–4 scale)
A2	Previous diagnosis of essential thrombocythemia or polycythemia vera
B1	New palpable splenomegaly or increase in spleen size of >5 cm
B2	Unexplained anemia with 20 g/l decrease from baseline hemoglobin
B3	Leukoerythroblastic blood film.
B4	Teardrop red cells
B5	Constitutional symptoms†
B6	Histological evidence of extramedullary hematopoiesis

Diagnosis requires A1 + A2 and any two B criteria.
†Only drenching night sweats, weight loss >10% over 6 months, unexplained fever (>37.5°C) or diffuse bone pains (no other symptoms are included).

occurs due to a disease-related complication or progression to acute myeloid leukemia.

Symptoms may be heterogeneous and include both constitutional symptoms like fatigue, cachexia, pruritus, bone pain, fever and symptoms more directly related to the consequences of massive splenomegaly, which include pain, early satiety, splenic infarction and dyspnea. The degree of severity of symptoms of MF have been assessed and reported to be similar to those of advanced cancer [12].

The median survival for patients with PMF ranges between 2 and 15 years and is linked to a number of risk factors [13]. The survival of patients with PET-MF or PPV-MF is unclear and currently the prognostic risk scores outlined below have not been adequately or prospectively validated in this group of patients. Despite this lack of validation, we and others use them in patients with PET-MF and PPV-MF but note the risk of inaccuracy inherent in doing so.

There have been many prognostic scoring systems for MF. Currently those used in practice are the International Prognostic Scoring System (IPSS) at the time of diagnosis [13] and the Dynamic IPSS (DIPSS) [14,15] or DIPSS plus [16] during the course of the disease. The IPSS comprises the following five risk factors for estimating survival from time of diagnosis: age >65 years, hemoglobin level <100 g/l, leukocyte count >25 × 10^9/l, circulating blasts ≥1% and presence of constitutional symptoms. The presence of no, one, two, and three or more adverse factors define low, intermediate 1, intermediate 2 and high-risk disease with median survivals of 11.3, 7.9, 4 and 2.3 years, respectively [13]. With the use of the same prognostic variables, IPSS was later modified to DIPSS for use at any time during the disease course [15]. Most recently, DIPSS was upgraded to DIPSS-plus by the incorporation of three additional IPSS/DIPSS-independent risk factors including red cell transfusion need, platelet count <100 × 10^9/l and unfavorable karyotype [16]. The latter includes complex karyotype or one or more of the following abnormalities that include trisomy 8, monosomy 7/7q-, isochromosome (17q), inv (3), deletion 5/5q-, 12p- or 11q23 rearrangement. These data are all summarized in **Box 1 & Table 3**. An advantage of DIPSS plus is that it allows the identification of very low-risk patients and very high-risk patients when compared with IPSS or DIPSS. These scores are especially important for therapeutic decisions that include allogeneic stem cell transplantation (SCT), the only curative approach that still carries a risk of morbidity and mortality even with the newest reduced intensity conditioning regimens.

Recently, a molecular risk score based on the presence of any one of the mutations in ASXL1, EZH2, IDH 1 or 2 and SRSF2 has been proposed by the group of Alessandro Vannucchi [17] to delineate patients within IPSS prognostic groups with even worse predicted survival due to the occurrence of acute leukemia. If this score is validated it will be of importance in several ways; for example, identifying ultra-high-risk patients for upfront or early therapy with SCT or low-risk patients bearing these mutations who may have a significantly increased risk of leukemia and be suited to experimental therapies or studies designed at reducing the risk of leukemia.

Treatment strategies

MF remains an incurable disease for patients who are not successful recipients of SCT because no other medical intervention, until recent data with ruxolitinib became available (see below), had been shown to improve survival. Therefore, treatment is supportive and aimed at alleviating symptoms. According to the recommendations from the European LeukemiaNet, "the main goals of therapy in PMF are prolongation of survival and, if possible, also cure, which is currently only achieved by SCT. If prolongation of survival or cure is not possible, symptom-orientated palliation and quality of life are the main goals" [18].

In our practice therefore, we aim to identify patients who might be suitable for SCT early in the course of the disease and then identify the specific needs of patients, individualizing

therapy. This may include watchful wait for some asymptomatic low-risk patients (Figure 2). It is always important to explain to a patient the nature of MF and what symptoms and signs to watch out for, prognosis and the risks of disease progression. Common additional questions from patients in our experience are:

- 'Is this disease inherited?'
- 'Is there a difference between patients who do or do not have the *JAK2* V617F mutation?'
- 'Will a specific diet, exercise or homeopathic remedy help?'

Response to treatment is naturally important to assess and there are international criteria that may be utilized in this setting [19,20]; however, these require validation and may be problematic; for example, when a therapy induces anemia but responses in other categories, these criteria are not particularly useful and in clinical practice we rarely utilize them. New criteria for response and progressive disease are urgently required for MF.

Treatment of anemia

Anemia (disease but not treatment related) is the strongest adverse risk factor for prognosis in MF and it can be the most difficult problem to treat [13]. Blood transfusion is a standard therapy for symptomatically anemic patients and the transfusion target should be assessed individually and kept under review. Regular transfusions will eventually lead to iron overload, although it remains unclear what the potential for this to lead to toxicity and end-organ damage is and how relevant that may be for the majority of patients. We would mandate iron chelation for current or future SCT candidates and consideration for other patients. Other modalities for treating anemia that we utilize include recombinant erythropoietin (rEPO), anabolic steroids and thalidomide or similar agents.

Erythropoietin

In an analysis of 20 anemic MF patients treated with rEPO, responses were seen in 45% of cases but only maintained long term in 20% [21], with responses to rEPO being more likely in transfusion-independent patients with higher baseline hemoglobin. A pooled analysis of this 20-patient series with 31 patients from the literature demonstrated an overall rEPO response rate of 55% (31% complete response [CR]). with a median duration of 12 months [21]. In our own practice we rarely use rEPO unless the patient has chronic kidney disease or an endogenous EPO level of less than 125 IU/l. There has been some controversy in the field regarding whether the use of erythropoiesis-stimulating agents worsens prognosis, however, this is widely held not to be the case [18].

Box 1. Prognostic systems used for myelofibrosis in our practice: prognostic variables

IPSS or DIPSS score

1 point each, hemoglobin = 2 in the DIPSS score

- Age >65 years
- Constitutional symptoms (only fever, sweats or weight loss)
- Hemoglobin <100 g/l
- Leukocyte count >25 x 10^9/l
- Circulating blasts ≥1%

DIPSS plus

Add 1 point in addition to the DIPSS risk group† for:
(these are low = 0; intermediate 1 = 1, intermediate 2 = 2 and high risk = 3)

- Platelet count <100 x 10^9/l
- RBC transfusion need
- Unfavorable karyotype +8, _7/7q_, i(17q),inv (3), _5/5q_, 12p_, 11q23 rearrangement

†Note that this is the risk group not the sum of points.
DIPSS: Dynamic International Prognostic Scoring System; IPSS: International prognostic scoring system; RBC: Red blood cell.

Androgens

Danazol, a synthetic attenuated androgen, has found increasing favor as the first-line androgen of choice in MF for the management of anemia. This agent has been shown to have the additional

Table 3. Prognostic systems used for myelofibrosis in our practice: prognosis derived from variables.

Risk group	Predictors (n)	Median survival (years)
IPSS		
Low	0	11.3
Intermediate 1	1	7.9
Intermediate 2	2	4.0
High	>3	2.3
DIPPS		
Low	0	Not reached
Intermediate 1	1 or 2	14.2
Intermediate 2	3 or 4	4
High	5 or 6	1.5
DIPPS plus		
Low	0	15.4
Intermediate 1	1	6.5
Intermediate 2	2–3	2.9
High	>4	1.3

DIPSS: Dynamic International Prognostic Scoring System; IPSS: International Prognostic Scoring System.

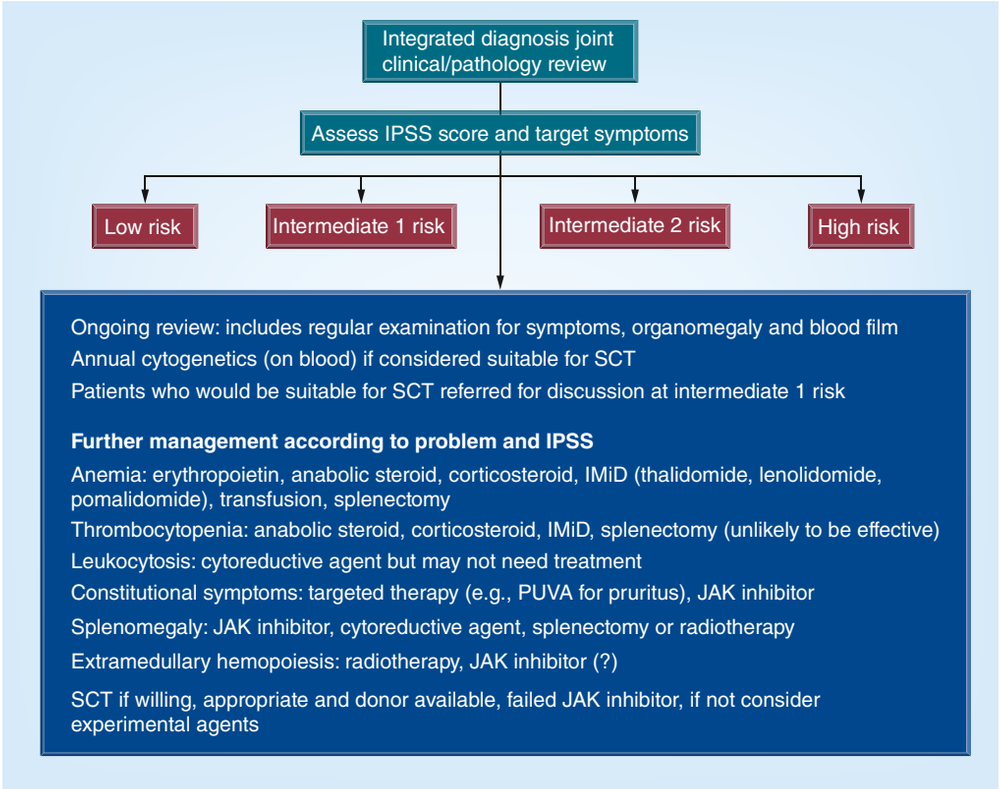

Figure 2. Management of myelofibrosis.
IMiD: Immune-mediated inflammatory disease; IPSS: International Prognostic Scoring System; PUVA: Psoralen plus UVA light; SCT: Stem cell transplantation.

benefits of reducing spleen size in a proportion of patients. In the study by Cervantes *et al.*, patients were initially commenced on danazol at a dose dependent on body weight: 600 mg daily for those weighing ≤80 kg and 800 mg daily for those weighing >80 kg [22]. This dose was continued for a minimum period of 6 months before evaluating response. Those achieving a favorable response were maintained on danazol at a reduced dose of 400 mg daily for a further 6 months before the dose was titrated down to the minimum required to maintain a response (200 mg daily). Although the side effects are well recognized, including fluid retention, increased libido, hirsutism, deranged liver function tests and hepatic tumors, only two responders in this study by Cervantes *et al.* discontinued treatment because of toxicity (one from cholestatic hepatitis and one from prostatic adenocarcinoma). Based on these observations, we treat for 6 months before assessing the response and monitor all patients receiving danazol with liver function tests monitored at least monthly during initial therapy and a liver ultrasound performed for hepatic malignancy periodically. Males are screened for prostate cancer before therapy and during treatment. Other synthetic androgens have also been used in this setting, and in a patient with intolerance or lack of response to one androgen, a second similar agent such as oxymethalone may be useful, although we would rarely use this agent [23].

■ Thalidomide & other immunomodulating agents

Thalidomide has some efficacy in managing anemia with some reponses in thrombocytopenia and splenomegaly as has been reported in several studies (summarized Thapaliya *et al.* [24]). In our own practice we usually use this agent in combination with prednisolone and, due to the side-effect profile of this agent, would not usually select it for first-line management of anemia [25]. In the very infrequent PMF patient with del(5q31)-associated anemia, lenalidomide is the recommended first-line therapy because significant improvement, with resolution of

anemia and occasionally evidence of molecular remission, has been reported [26]. There is much interest in the potential for pomalidomide to ameliorate anemia; a number of Phase II studies have been reported [27–29] and a Phase III study, RESUME, is due to report soon and has the potential to significantly influence the way anemia is managed for these patients.

Splenomegaly & extramedullary hemopoiesis

The main approaches for the treatment of symptomatic splenomegaly are medical or surgical. JAK inhibitors, at present primarily ruxolitinib, will radically alter the way splenomegaly is managed. At the time of writing, ruxolitinib was not readily available in the UK and since we wished to discuss all efficacy data with this agent in one section of this article we only refer to it here briefly. Medical treatment remains the treatment of choice for most patients with symptomatic splenomegaly. The first choice is hydroxycarbamide (HC; hydroxyurea), which usually produces modest responses at higher doses that are not easily tolerated because of newly developed or exacerbated cytopenias. Greater than 25 and 50% reductions in spleen size have been reported in up to 35 and 17%, respectively, of the patients treated with HC [30]. In the randomized COMFORT-II study (described later), none of the 73 patients in the 'best-available treatment' (BAT) arm, of whom 60% received HC, achieved a sustained >35% spleen volume reduction (which equals a 50% reduction in palpable spleen) [31]. Ruxolitinib and other JAK inhibitors are likely to surpass HC for first-line treatment of symptomatic splenomegaly (see later).

Busulfan, and occasionally melphalan, are used in older subjects who do not tolerate or respond to HC, but they are even more myelosuppressive. As discussed earlier, responses in splenomegaly with low-dose thalidomide (50 mg/day) are infrequent (<20%). Lenalidomide has also been shown to produce a response rate of 33% in a study that included some patients who had failed on prior thalidomide therapy. Neither of these agents would be frequently used in our practice. In cases of massive, refractory splenomegaly, monthly intravenous cladribine (2-CdA; 2-chlorodeoxyadenosine) courses produced up to 50% responses, with severe but reversible cytopenias being the main toxicity [32]. Both standard and pegylated preparations of interferon-α appear to have little clinical effect in reducing splenomegaly [33] and, as a result, their use is not generally recommended; in our practice we tend to reserve this agent for younger patients with early-stage disease. The agents listed above do, however, have a role as myelosuppressive agents (see later).

The place of splenectomy in the management of MF is well established. However, we and others consider that routine splenectomy is inappropriate and the procedure should be restricted to carefully selected patients with refractory hemolysis or anemia, severely symptomatic splenomegaly, significant splenic infarction, severe portal hypertension and severe hypercatabolic symptoms. Even in the best units, splenectomy is associated with morbidity and mortality rates of approximately 31 and 9%, respectively [34]. Those patients undergoing splenectomy need to be well aware of the risks and very carefully assessed preoperatively with meticulous postoperative care. Hepatic extramedullary hematopoiesis leading to rapid hepatic enlargement is an unusual but well-recognized complication following splenectomy, as is the increased thrombotic risk. It is extremely rare for us to resort to splenectomy in our clinical practice.

Radiotherapy is a valuable alternative to splenectomy in patients with symptomatic splenomegaly and an adequate platelet count (>50 × 10^9/l) and in whom surgery is deemed unsuitable. In the Mayo Clinic experience, a median radiation dose of 277 cGy was administered in a median of 7.5 fractions. Reduction in spleen size was noted in the majority of cases and lasted for a median of 6 months, although 44% experienced cytopenias, of which 13% were fatal [35].

Our own practice is to use even lower dosing fractions with caution. Low-dose irradiation remains the treatment of choice for extramedullary hematopoiesis at other sites, including involvement of the peritoneum and pleura with resultant ascites and pleural effusions, respectively. The role of JAK inhibitors (see later) in management of extra medullary hematopoiesis outside the spleen or liver, is of great potential interest.

Management of constitutional symptoms

Multiple symptoms, such as fatigue, weakness, abdominal pain, cachexia, weight loss, pruritus, night sweats and bone pain are common in patients

with PMF, particularly but not exclusively, in those with advanced disease. The debilitating symptoms of MF are thought to be driven by the combined effects of massive splenomegaly and elevated levels of proinflammatory cytokines. Quality of life scores for PMF patients have been reported to be equivalent to those for advanced metastatic cancer [12]. The efficacy of conventional therapy in moderating these symptoms is poor. Evidence for a major benefit of JAK inhibitors in this aspect is now overwhelming, thus far however this has only been tested for patients with the combination of splenomegaly and constitutional symptoms (see later). Efficacy of conventional therapies against severe constitutional symptoms is modest at best, although in one study, up to 80% of the patients receiving HC had a response in constitutional symptoms [30]. Results from the randomized COMFORT-II study indicated that none of the patients in the BAT arm presented measurable improvements in symptoms, as measured by the EORTC QLQ-C30 or FACT-Lym scores [31]. Low-dose prednisone may sometimes produce a feeling of well-being, but the effect is usually modest and transient in our experience and accompanied by the anticipated side effects of corticosteroid use.

Myelosuppression

Myelosuppressive therapy in PMF is not curative and there are relatively few published series, most of which are small, nonrandomized and incorporate different definitions of response. Nevertheless, indications for myelosuppression also include the control of symptoms related to hypercatabolism (fever, night sweats, fatigue, weight loss and bone pains), splenomegaly and hepatomegaly.

As discussed earlier, Martinez-Trillos *et al.* have reported that HC is an effective and relatively well-tolerated therapy for the control of the hyperproliferative symptoms of PMF [30]. Data from the Primary Thrombocythemia 1 study [36] and the Swedish Myeloproliferative Disorder Study Group [37] have suggested that anagrelide treatment, when compared with HC, may be associated with an increase in reticulin grade and therefore, this agent is only used with caution in patients with MF in our clinical practice.

More recently, two small, retrospective, observational studies have shown promising results with interferon in PMF patients. First, a group from Cornell University (NY, USA) reported that the use of IFN-α2b early in the disease course, starting at a very low dose (0.5–1.0 million units three times per week), can slow disease progression, with some patients exhibiting regression of marrow fibrosis [38]. A second study observed clinically significant efficacy of pegylated-IFN-α2a in PMF, with 44% of patients experiencing complete or major responses, with six out of eight patients normalizing hemoglobin levels (including two out of three transfusion-dependent patients) [33]. In both studies, patients with advanced disease and massive splenomegaly showed a lower response rate. We generally only use interferon in younger patients with earlier stage MF but feel this is an agent of great potential interest.

Allogeneic SCT

Allogeneic hematopoietic SCT is the only curative treatment for patients with MF at present. Due to the significant morbidity and mortality associated with SCT, divergent opinions have emerged about the application of SCT in MF. Significant regimen-related toxicities, graft failure and graft-versus-host disease are major barriers to the success of SCT in MF. Use of reduced-intensity conditioning has helped to expand the applicability of SCT to older patients with MF. However, in overall disease management, the option of SCT is applicable only to a small proportion of MF patients. A large proportion of patients are not in the transplant age group at the time of diagnosis. Among younger patients, suitable related or unrelated donors are found in approximately 40–50% of cases.

Data from the most recent studies suggest that progression-free survival in the range of 40–50% at 3 years can be expected with SCT (as reviewed in [39]), thus it remains a valid option for patients in the transplant age group with adequate performance status and without any prohibitive comorbidities. Splenectomy is not routinely recommended in preparation for SCT. However, is it reasonable to explore the safety and efficacy of novel drugs, such as JAK and mTOR inhibitors, which produce rapid spleen shrinkage and improvement of constitutional symptoms in the immediate pretransplantation setting, as we have discussed in a recent review [39]. A study on MPD RC114 will shortly be evaluating the safety of ruxolitinib in the pretransplant setting. The

recommended indications for transplantation in our practice are expected survival of less than 5 years, transfusion dependency, or an increased risk of leukemic transformation. The availability of a fully matched sibling donor in general would lead us to conduct a transplant earlier (intermediate 2 or intermediate 1 with anemia, transfusion or rising blasts).

JAK inhibitor therapy

The availability of JAK inhibitor therapy is undoubtedly the most important development in MF in recent years. For this reason we are discussing these agents separately, although we have referred to them above. The first-in-class JAK1/2 inhibitor, ruxolitinib, has been approved by the US FDA and Health Canada, as well as the EMA for patients with MF. Other JAK inhibitors are at various stages of clinical development. Ruxolitinib will be the most widely available JAK1/2 inhibitor for routine clinical use in patients with MF in the near future, and other agents are likely to be approved in due course.

■ Ruxolitinib

Regulatory approval was based on the results of two pivotal randomized Phase III trials: COMFORT-I in the USA, Canada and Australia [40] and COMFORT-II in Europe [41]. The trials enrolled patients with PMF, PET-MF or PPV-MF with intermediate 2 risk or high-risk disease as assessed by IPSS, and platelet count >100 × 10^9/l. In COMFORT-I, 309 patients were randomized 1:1 to ruxolitinib or placebo, whereas in COMFORT-II, 219 patients were randomized 2:1 to ruxolitinib or BAT. The primary end point of both studies was a 35% reduction in spleen volume, which in Phase I/II studies was shown to be an equivalent of 50% reduction in spleen length by palpation from the costal margin.

In COMFORT-I, 41.9% of ruxolitinib patients had a ≥35% reduction in spleen size at 24 weeks versus 0.7% of placebo patients ($p < 0.001$); 67.0% maintained this response for ≥48 weeks. There was a >50% improvement in symptom score at 24 weeks in 45.9% of ruxolitinib patients versus 5.3% of placebo patients ($p < 0.001$). There was no significant difference in response among patients with or without the *JAK2* V617F gene mutation.

The *JAK2* V617F allele burden was reduced by 21.5% at week 48. The survival analysis at 51 weeks' median follow-up demonstrated increased mortality in the placebo arm (15.6 vs 8.4%; $p = 0.04$). In COMFORT-II, 28% of ruxolitinib patients achieved the primary end point of ≥35% reduction in spleen size by MRI at week 48 compared with 0% with BAT ($p < 0.001$); 80% maintained the response at a median 12-month follow-up. There was no difference in overall survival or leukemia-free survival, and no change was observed in bone marrow pathology. There does not appear to be any difference in leukemic transformation in patients treated with ruxolitinib when compared with control arms in the two trials. A *post-hoc* analysis of data from both trials found that there was no difference in quality of life outcomes between the placebo arm of the COMFORT-I trial and the BAT arm of the COMFORT-II trial [42].

Overall, ruxolitinib is well tolerated, with the main toxicity being hematological. In COMFORT-I, grade 3–4 hematological effects occurring more frequently with ruxolitinib included anemia (45.2 vs 19.2%), thrombocytopenia (12.9 vs 1.3%) and neutropenia (7.1 vs 2.0%). On average, ruxolitinib-treated patients had a hemoglobin nadir of 15–20 g/l below baseline at 8–12 weeks, stabilizing at an average reduction of approximately 10 g/l at 24 weeks. Some data suggest that rEPO may be useful to treat anemia in patients treated with ruxolitinib [43], and we do use these agents in combination in our clinical practice. Thrombocytopenia led to dose modification in 41% of patients in COMFORT-II. Responses to ruxolitinib were not sustained following treatment discontinuation. Symptom scores returned to baseline within 1 week of discontinuation of ruxolitinib; there was no evidence of severe inflammatory syndrome after ruxolitinib withdrawal.

At the 2012 American Society of Hematology meeting, both COMFORT studies reported updated results. The long-term follow-up analysis of COMFORT-I (presented by Verstovsek *et al.* [44]), showed that 100 of the 155 patients randomized to the ruxolitinib arm (64.5%) remained on treatment after a median follow-up of 102 weeks. Cervantes *et al.* presented 2-year data from the COMFORT-II study [45]. At the time of analysis, the median follow-up was 112 weeks (ruxolitinib: 113 weeks; BAT: 108 weeks), and the median duration

of exposure was 83.3 weeks (ruxolitinib: 111.4 weeks; BAT: 45.1 weeks). Overall, 73.3% of patients (107 out of 146) in the ruxolitinib arm entered the extension phase and 55.5% (81 out of 146) of those originally randomized to ruxolitinib remained on treatment at time of the analysis. Among patients randomized to the BAT arm, 61.6% (45 out of 73) crossed over to receive ruxolitinib, and the majority of them were still receiving ruxolitinib, confirming that the drug is well tolerated.

In both studies spleen volume reductions of ≥35% were sustained with continued ruxolitinib therapy. In COMFORT-I, mean spleen volume reduction in patients randomized to ruxolitinib was 31.6% at week 24 and has remained stable with additional follow-up up to week 96. In those patients who achieved a ≥35% reduction in spleen volume, the median response duration was 108 weeks. The probabilities of maintaining the spleen response on COMFORT-II for at least 48 and 84 weeks are 75% (95% CI: 61–84%) and 58% (95% CI: 35–76%), respectively, and the median duration of response in this study has not yet been reached. Concerning patient-reported outcomes, long-term follow-up of COMFORT-I demonstrates that ruxolitinib treatment was associated with durable clinically significant improvements in the Global Health Status/QoL and the five functional domains of the EORTC QLQ-C30 questionnaire [43].

No new adverse events were reported with more than 2 years of ruxolitinib treatment. Anemia and thrombocytopenia are anticipated and not infrequent with ruxolitinib; data indicated a lower incidence of both after week 48 (anemia: 22.6%; thrombocytopenia: 25.2%) and the majority were grade 1/2. Additionally, as demonstrated at the time of the primary analysis for each of the COMFORT studies, anemia and thrombocytopenia rarely led to treatment discontinuation (<1% of patients in any treatment group) and were manageable with dose modifications and/or transfusions. Indeed, in the COMFORT-I study update, the proportion of patients receiving red blood cell transfusions in the ruxolitinib arm decreased to the level seen among patients receiving placebo by week 36 and remained stable thereafter. Of note, there were no new reports of leukemic transformation in either study and no specific patterns of adverse events or reports of a withdrawal syndrome after discontinuation of ruxolitinib were observed with longer follow-up.

Concerning survival, the COMFORT-I investigators continue to report that, despite the majority of patients switching to ruxolitinib from placebo, earlier treatment with ruxolitinib is associated with a survival advantage. Since the last report of COMFORT-II (median: 61.1 weeks), an additional nine and 12 deaths were reported in the ruxolitinib and BAT arms, respectively, resulting in a total of 14% (20 out of 146) and 22% (16 out of 73) of patients overall; the median survival time has not yet been reached for both arms. For the first time in COMFORT-II, patients randomized to ruxolitinib showed longer overall survival than those randomized to BAT (HR: 0.51; 95% CI: 0.27–0.99; log-rank test, p = 0.041). In COMFORT-II, the ruxolitinib and BAT arms may not have separated early in the Kaplan–Meier curve because a considerable number of patients in the BAT arm were censored prior to 48 weeks (27.4% of patients in the BAT arm versus 14.4% of patients in the ruxolitinib arm). This means that they were considered alive in the absence of any further information. This factor, along with the 2:1 randomization, may bias the data in favor of BAT. However, despite these factors and the crossover of a majority of BAT patients to ruxolitinib, there was an apparent survival benefit favoring ruxolitinib in this intent-to-treat analysis. The overall survival advantage for ruxolitinib-treated patients, despite the limitations described earlier, would suggest that even the relatively short period of additional treatment for the patients initially randomized to ruxolitinib (6 months in COMFORT-I and 1 year in COMFORT-II) may have had a significant effect on survival.

These data will be followed by further updates of these studies next year. At American Society of Hematology (ASH) we also heard that allele burden reductions with ruxolitinib in COMFORT-II are relatively modest [46]. Long-term data concerning marrow histology and other data, such as acquisition of new mutations, are awaited.

In clinical trials, a dose-adjustment strategy for ruxolitinib based on platelet count was used to minimize toxicity: the starting dose was 20 mg twice a day (b.i.d.) for platelet count >200 × 10^9/l and 15 mg b.i.d. for platelet count 100–200 × 10^9/l. A dose of 15 mg b.i.d. may

also be considered in transfusion-independent patients, who may have difficulty tolerating a drop in hemoglobin of 20 g/l. In our personal experience, improvement of constitutional symptoms can be observed even at lower doses (5 mg b.i.d.). Dose reduction should be considered for patients receiving ruxolitinib 15 or 20 mg b.i.d. if the platelet count declines below 100. When treatment interruption is required, dose tapering is advised. Dose increases in increments of 5 mg b.i.d. can be considered on a monthly basis to a maximum dose of 25 mg b.i.d. in patients with inadequate response if no significant hematological toxicity occurs. We monitor blood counts at least every 2 weeks over the first 4–6 weeks or longer until counts are stabilized.

Both COMFORT-I and COMFORT-II enrolled patients with a platelet count >100 × 10^9/l. Approximately 25–30% of MF patients in need of JAK inhibitor therapy may have a platelet count <100 × 10^9/l. Preliminary results of ongoing trials suggest that a starting dose of 5 mg b.i.d. followed by a dose-escalation strategy is tolerable and efficacious for patients with platelet counts of 50–100 × 10^9/l [47,48]. Currently, we seek to use JAK inhibitors either in a clinical trial or when they can be obtained as a priority for patients with troublesome symptoms or hepato- or spleno-megaly.

Other JAK inhibitors

A range of other JAK inhibitors are at various stages of development; we have referred to three that we have had experience of using in our clinical practice and where the data were updated at the recent ASH meeting.

■ SAR302503

Talpaz and colleagues recently reported further data evaluating the JAK2 inhibitor SAR302503, presenting the results of 31 MF patients randomized in a Phase II study to doses of 300, 400 and 500 mg per day [49]. All patients had completed week 12 at the time of analysis. The median percentage reduction in spleen volume from baseline ranged from 30.1 to 41.8%, with a dose-dependent increase; overall, 63.6% of patients receiving 500 mg achieved ≥35% reduction in spleen volume. There appeared to be a correlation between pharmacokinetics data and spleen response. Reduction of MF-related symptoms appears to be similar to other JAK2 inhibitor trial outcomes. Concerning safety, the most common nonhematologic adverse events were gastrointestinal and did not lead to permanent drug discontinuation; anemia occurred but grade 3/4 thrombocytopenia was infrequent. This agent has previously been reported to be associated with allele burden and bone marrow fibrosis grade reductions [50]. These data suggest that this drug may be of great interest in the management of MF; the results of a Phase III study (JAKARTA) are awaited. Meanwhile a study utilizing this agent in patients intolerant or resistant to ruxolitinib (JAKARTA-2) is recruiting, and our personal experience in patients unable to tolerate ruxolitinib due to thrombocytopenia is encouraging.

■ CYT387

CYT387 is a small molecule ATP-competitive aminopyrimidine derivative with potent JAK kinase inhibitory activity [51,52]. Pardanani *et al.* presented updated data at ASH 2012 with this agent [53]. A sizable cohort of 166 subjects were enrolled and the median duration of follow-up was 16.1 months (range: 0.7–31.0 months). Updated safety and efficacy results were presented when patients reached a minimum of 9 months on study. Particular novel data of interest with this compound are transfusion independence responses, which were observed in more than half of the red blood cell transfusion-dependent subjects, with a maximal transfusion-free period exceeding 2 years and ongoing. In addition, the percentage of all subjects requiring red blood cell transfusions substantially decreased over the treatment period. As previously reported, treatment with CYT387 resulted in rapid and sustained reductions in splenomegaly, now with a maximal response duration approaching 2 years; symptomatic responses were also encouraging, yet the methodology used in this trial make symptomatic response difficult to compare with other trial reports. Concerning safety, the most common treatment-related adverse events were thrombocytopenia, peripheral neuropathy, dizziness, diarrhea, nausea, and headache. Treatment-related peripheral neuropathy with this agent was reported as sensorial and mainly grade 1. There were no treatment-related deaths.

■ Pacritinib

SB1518 (pacritinib) is another potent inhibitor of the JAK1, JAK2 and TYK2 kinases. The

safety of SB1518 was tested in a Phase I study involving 43 patients who had failed therapy, including 36 with MF and seven with acute myeloid leukemia [54]. The dose-limiting toxicities were gastrointestinal toxicity and decline in performance status, occurring in patients receiving 600 mg daily. Of interest, this study, which was the only other study to use MRI imaging of the spleen volume, suggested that a 50% reduction by physical examination was equivalent to a 25% reduction in volume by MRI (contrasting with the ruxolitinib study, where this was equivalent to a 35% reduction in volume by MRI). After treatment with this agent, 57% of patients had at least a 25% reduction in spleen volume and a 40–65% improvement in symptoms (abdominal pain, bone pain, early satiety, inactivity, night sweats and pruritus) was observed at month 6 versus baseline. A Phase III study of SB1518 including patients with MF with low platelets and symptomatic splenomegaly is planned (PERSIST). Interestingly, in contrast with ruxolitinib, SB1518 did not cause any significant hematologic toxicity. This may be an important asset of this drug.

Other experimental strategies

■ Everolimus (RAD001)

Activation of the AKT/mTOR pathway occurs in MF. A Phase I/II study with everolimus, an mTOR inhibitor, in 39 high- or intermediate-risk PMF or PPV-MF/PET-MF subjects has recently been reported [55]. Responses were evaluated in 30 patients of Phase II. A total of 69 and 80% experienced complete resolution of systemic symptoms and pruritus. Response rate was 60% when European Myelofibrosis Network criteria were used (eight major, seven moderate and three minor responses) or 23% when International Working Group for Myelofibrosis Research and Treatment criteria (one partial response and six clinical improvements) were used. These results provide proof-of-concept that targeting the mTOR pathway in MF may be clinically relevant.

■ Panobinostat

LBH589 is a novel pan-deacetylase inhibitor that has demonstrated clinical activity in Phase I/II studies in patients with a variety of hematologic malignancies. Both Phase I/II studies with this agent identified reversible thrombocytopenia as the dose-limiting toxicity and reported evidence of clinical responses [56,57]. The Phase I study reported by Mascarenhas *et al.* is of particular interest as one subject achieved a near CR at 16 months with resolution of palpable splenomegaly, elimination of peripheral blood dacrocytes and leukoerythroblastosis, a 40 g/l increase in hemoglobin, improvement in overall marrow cellularity and megakaryocyte atypia with an increase in erythroid precursors and a significant reduction of reticulin/collagen fibrosis [56]. They concluded that low doses of LBH589 delivered for more than 6 months in patients with MF are capable of ameliorating symptoms, improving clinical features and reversing pathologic marrow changes. This is an agent of interest in this field and is currently being taken forward in clinical trials in combination with ruxolitinib.

■ Telomerase inhibition

Telomerase is upregulated in neoplastic progenitor cells and sustains indefinite replication. Imetelstat is a first-in-class, potent, specific inhibitor of telomerase that selectively distributes to bone marrow and inhibits thrombopoiesis. In a Phase II study reported at ASH 2012, this agent was tested in patients with ET who had failed or were intolerant to HC [58]. Concerning hematological response, 11 of 13 patients achieved a confirmed CR after a median of 6.1 weeks (range: 5.1–14.1 weeks). In addition, the reduction in *JAK2* V617F allele burden and cytokine-independent growth of colony-forming unit-megakaryocytes suggests that imetelstat has a relatively selective inhibitory effect on the growth of the neoplastic clone(s) that drive ET and has the potential to modify the underlying biology of myeloproliferative neoplasms. However, this drug was toxic, although it may merit testing in MF.

■ Combination therapy

Combining therapeutic modalities is standard practice for the management of the majority of hematological malignancies with the aim of improving response in the drive for cure and also in some circumstances to improve tolerability. An example of the latter that has already been discussed is the combination of rEPO and ruxolitinib, and the potential for combining JAK inhibition with SCT, with which we have had successful anecdotal experience. There are other therapies that could be usefully combined with JAK inhibitors or perhaps two agents with different modes of action. These have recently been reviewed, although the number of potential option is growing [59]. This

type of therapy is offered to patients for whom transplant or ruxolitinib is not an option or who have progressive disease despite these therapies.

Conclusion

The data from the Phase III studies with ruxolitinib marked a pivotal point in the therapy of patients with MF. Much, however, remains to be learnt and considered. As we have discussed, other JAK inhibitors and additional agents, both alone and in combination, are currently being assessed in MF and have the potential for great merit in improving the lives of patients with these diseases. However, long-term data with regard to both safety and efficacy of these new strategies are undoubtedly required. In parallel, better understanding of the pathophysiology of this complex disease, as well as robust standardized clinical criteria for diagnosis response and progression should be provided.

Future perspective

The therapeutic landscape for MF has radically shifted with the introduction of JAK inhibitors. However, we still need to understand what defines a response to these agents and how we should be use them. Options might include, for example, treating until there is 50% palpable spleen reduction or maximizing the dose to tolerability. In the future, we should have a better idea of the efficacy of the other JAK inhibitors and, perhaps, which types of patients do better with which drug. Studies will hopefully have begun in earlier phases of the disease, possibly in those identified by molecular stratification to be at higher risk.

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