How I treat myelofibrosis

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Abstract

It is currently assumed that myelofibrosis (MF) originates from acquired mutations that target the hematopoietic stem cell and induce dysregulation of kinase signaling, clonal myeloproliferation and abnormal cytokine expression. These pathogenetic processes are interdependent and also individually contributory to disease phenotype–bone marrow stromal changes, extramedullary hematopoiesis, ineffective erythropoiesis and constitutional symptoms. The molecular pathogenesis of MF is poorly understood despite a growing list of resident somatic mutations that are either functionally linked to JAK-STAT hyperactivation (e.g. JAK2, MPL and LNK mutations) or possibly involved in epigenetic dysregulation of transcription (TET2, ASXL1 or EZH2 mutations). Current prognostication in primary MF is based on the Dynamic International Prognostic Scoring System-plus (DIPSS-plus) model, which uses eight independent predictors of inferior survival to classify patients into low, intermediate-1, intermediate-2 and high-risk disease groups; the corresponding median survivals are estimated at 15.4, 6.5, 2.9 and 1.3 years. Such information is used to plan a risk-adapted treatment strategy for the individual patient, which might include “observation alone”, conventional or investigational (e.g. JAK inhibitors, pomalidomide) drug therapy, allogenic stem cell transplant using reduced or conventional intensity conditioning, splenectomy or radiotherapy. I will discuss these treatment approaches in the context of who should get what and when.
Terminology

What do I mean by myelofibrosis?

Bone marrow fibrosis, usually revealed by silver (reticulin fibrosis) or trichrome (collagen fibrosis) stains, can accompany a number of hematologic and non-hematologic conditions including myeloid or lymphoid neoplasms, metastatic cancer, autoimmune diseases, hyperparathyroidism, vitamin D deficiency, pulmonary hypertension, grey platelet syndrome, treatment with growth factors (e.g. thrombopoietin agonists), hereditary thrombocytosis (e.g. germline MPLS505N mutation), infections (e.g. AIDS, leishmaniasis) and exposure to toxic substances (e.g. thorium dioxide).\(^1\) When describing such occurrences, I prefer to use the phrase “with bone marrow fibrosis” instead of “with myelofibrosis”: for example, “myelodysplastic syndromes (MDS) with bone marrow fibrosis” rather than “MDS with myelofibrosis”. I use the term myelofibrosis (MF) exclusively in reference to the myeloproliferative neoplasm (MPN) that is classified by the World Health Organization (WHO) system as primary myelofibrosis (PMF),\(^2\) or the phenotypically-similar condition that develops in the setting of either polycythemia vera (post-PV MF) or essential thrombocythemia (post-ET MF).\(^3\) Also, given our current understanding regarding the clonal nature of MF,\(^4\) it is inaccurate to continue using alternative terms such as “agnogenic myeloid metaplasia” or “chronic idiopathic myelofibrosis”\(^5\).

Classification

W.H.O. all the way

The WHO classification system for hematopoietic tumors recognizes five categories of myeloid malignancies including AML, MDS, MPN, MDS/MPN overlap and PDGFR/FGFR1-rearranged myeloid/lymphoid neoplasms with eosinophilia (Table 1).\(^2\) The WHO MPN category includes eight subcategories: PV, ET, PMF, chronic myelogenous leukemia (CML), chronic neutrophilic leukemia, chronic eosinophilic leukemia-not otherwise specified, mastocytosis and MPN-unclassifiable. The first...
four were originally described by William Dameshek as “myeloproliferative disorders” and are therefore currently referred to as “classic” MPN (Table 1). “BCR-ABL1-negative MPN” is an operational term that is used in reference to PV, ET and PMF. Post-PV MF and post-ET MF are classified and diagnosed according to consensus criteria established by the International Working Group for MPN Research and Treatment (IWG-MRT). The IWG-MRT also recommends the use of the term “blast phase PMF” when describing leukemic transformation.

Pathogenesis

Clones, mutations and cytokines: what do they all mean?

MPN are stem cell-derived monoclonal (or possibly oligoclonal) hemopathies. Family studies and JAK2 SNP/haplotype analyses suggest genetic predisposition to MPN. Although the disease-initiating mutation in MF is not known, the majority of the patients harbors JAK2V617F and a minority MPL, LNK, CBL, TET2, ASXL1, IDH, IKZF1 or EZH2 mutations (Table 2). These mutations are neither disease-specific nor mutually-exclusive, and likely constitute secondary events with unpredictable clonal hierarchy. Activating JAK2 and MPL mutations and LNK loss-of-function result in constitutive JAK-STAT activation and induce MPN-like disease in mice. Therefore, it was appropriate to pursue the therapeutic value of anti-JAK ATP mimetics. TET2, ASXL1 and EZH2 mutations are suspected of playing a role in epigenetic dysregulation of transcription. IDH mutations result in the formation of oncoproteins that might promote disease progression. The individual frequency of the aforementioned mutations in MF (Table 2), save for JAK2V617F, is too low to be overexcited about their utility as drug targets.

Clonal myeloproliferation in MF is accompanied by a secondary inflammatory state characterized by bone marrow stromal changes and abnormal cytokine expression. Myeloid cell-derived transforming growth factor-β (TGF-β), platelet derived growth factor, fibroblast growth factor-
b and vascular endothelial growth factor have all been implicated as mediators of bone marrow fibrosis, osteosclerosis and angiogenesis in MF.4 The abnormal release of cytokines, chemokines and extracellular matrix metalloproteinases (or their inhibitors) might be enhanced by a pathologic cell-cell interaction that involves megakaryocytes, monocytes and neutrophils26 and contributes to MF-associated abnormal peripheralization of CD34-positive myeloid progenitors27 and endothelial cells.28 Plasma levels of proinflammatory cytokines are elevated in MF and might be pathogenetically linked to disease-associated constitutional symptoms and cachexia.21 In addition, a recent study suggested significant associations between increased IL-8, IL-10, IL-15 or IL-2R and inferior overall and leukemia-free survival in PMF.29 These observations lend to the possibility that inflammatory mediators might affect survival in MF by either accelerating death from comorbid conditions or promoting clonal evolution.

**Diagnosis**

*How do I diagnose PMF, post-PV/ET MF and prefibrotic PMF?*

Current diagnosis of PMF is based on WHO-criteria and includes clinical, morphologic, cytogenetic and molecular assessment (Table 3).30 The diagnosis of post-PV or post-ET MF is according to IWG-MRT criteria (Table 3).3 In all three MF variants, typical laboratory features include anemia (microcytic in ~28% of PMF cases),31 peripheral blood leukoerythroblastosis, dacyrocystosis, leukocytosis/thrombocytosis, increased LDH, excess circulating blasts or CD34-postive cells and bone marrow fibrosis, osteosclerosis and angiogenesis. Occasionally, overt bone marrow fibrosis might be absent (i.e. prefibrotic PMF) and, in the presence of thrombocytosis, a spurious diagnosis of ET is made. The possibility of prefibrotic PMF, as opposed to ET, should be considered in the presence of persistently increased serum LDH, anemia, leukoerythroblastosis, increased circulating CD34-positive cell count and marked splenomegaly.32 It is underscored that the distinction between ET and
prefibrotic PMF is clinical relevant since both overall and leukemia-free survival are significantly inferior in the latter.\textsuperscript{32} However, there is currently no evidence to suggest a different treatment approach for patients with prefibrotic PMF and thrombocytosis; I manage them the same as if they had ET.

The differential diagnosis of PMF should also include bone marrow fibrosis associated with non-neoplastic or other neoplastic conditions including metastatic cancer, lymphoid neoplasm or another myeloid malignancy, especially CML, MDS, CMML or AML. The presence of \textit{JAK2} or \textit{MPL} mutation, with a combined mutational frequency of approximately 70\%, reliably excludes reactive bone marrow fibrosis or a non-myeloid malignancy.\textsuperscript{33} The absence of \textit{BCR-ABL1} excludes the possibility of CML. MDS or CMML should be considered in the presence of dyserythropoiesis/dysgranulopoiesis or peripheral blood monocytosis (\(>1 \times 10^9/L\)), respectively.\textsuperscript{34} In this regard, the presence of +9 or 13q- cytogenetic abnormality favors a PMF diagnosis.\textsuperscript{35} In contrast, \textit{JAK2V617F} can occur in both MDS and CMML, although infrequently,\textsuperscript{36} and is therefore not very useful in distinguishing one myeloid malignancy from another. “Acute myelofibrosis”, “acute panmyelosis with myelofibrosis” and “acute megakaryoblastic leukemia” are terms used to describe a biologically-aggressive myeloid malignancy that presents acutely with bone marrow fibrosis, pancytopenia and usually no splenomegaly.\textsuperscript{37} Such cases should be managed as AML regardless of which term one uses to describe them.

\section*{Risk Stratification}

\textit{Prognosis dictates treatment}

The International Prognostic Scoring System (IPSS)\textsuperscript{38} uses five risk factors for estimating survival from time of diagnosis: age \(>65\) years, hemoglobin \(<10\) g/dL, leukocyte count \(>25 \times 10^9/L\), circulating blasts \(\geq1\%\) and presence of constitutional symptoms.\textsuperscript{38} The presence of 0, 1, 2 and \(\geq3\)
adverse factors defined low, intermediate-1, intermediate-2 and high risk disease with median survivals of 11.3, 7.9, 4 and 2.3 years, respectively. Using the same prognostic variables, IPSS was later modified to Dynamic IPSS (DIPSS) for use at any time during the disease course. Most recently, DIPSS was upgraded to DIPSS-plus (Figure 1) by the incorporation of three additional IPSS/DIPSS-independent risk factors including red cell transfusion need, platelet count <100 x 10^9/L and unfavorable karyotype; the latter includes complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p- or 11q23 rearrangement. The 8 DIPSS-plus risk factors are used to define low (no risk factors), intermediate-1 (one risk factor), intermediate-2 (two or 3 risk factors) and high (four or more risk factors) risk groups with respective median survivals of 15.4, 6.5, 2.9 and 1.3 years (Figure 1). Leukemic transformation was predicted by the presence of unfavorable karyotype or platelet count <100 x 10^9/L.

The presence or absence of JAK2, TET2 or IDH mutations has not been shown to affect either survival or leukemic transformation in PMF. Instead, nullizygosity for JAK2 46/1 haplotype and low JAK2V617F allele burden have been shown to be detrimental for survival. Similarly, increased plasma levels of IL-8, IL-10, IL-15 or IL-2R have recently been associated with poor survival that was not accounted for by conventional risk categorization. In other words, our current ability to accurately estimate prognosis in the individual patient with PMF is better than ever and will probably continue to improve with time.

**Risk-adapted Therapy**

*Who gets what?*

Current drug therapy in PMF is neither curative nor essential for survival. Similarly, it is not clear if the application of allogeneic stem cell transplantation (allo-SCT), with its attendant risk of death or chronic morbidity from graft versus host disease (GVHD), has had a favorable or unfavorable
net effect. Therefore, one must first determine if a particular patient needs any form of therapy at all, and if so, carefully select the treatment strategy with the best chance of inducing disease control without compromising life expectancy (Figure 2).

**How I manage low or intermediate-1 risk patients**

According to the DIPSS-plus prognostic model, the respective median survival of low or intermediate-1 risk patients exceeds 15 and 6 years and even longer for patients below age 65 years. Therefore, the risk of allo-SCT-associated mortality and morbidity is not justified in such patients and it is also not prudent to subject them to investigational drug therapy considering the limited information regarding long-term safety of new therapeutic agents. Similarly, there is no evidence to support the value of conventional drug therapy in asymptomatic patients with low or intermediate-1 risk disease. Therefore, I prefer a “watch and wait” treatment strategy in such instances, regardless of patient age.

Low risk patients might occasionally experience symptoms associated with disease manifestations that do not necessarily affect DIPSS-plus risk stratification: splenomegaly, non-hepatosplenic extramedullary hematopoiesis, EMH-associated pulmonary hypertension, fatigue, bone (extremity) pain, pruritus or thrombocytosis with a thrombosis history. Intermediate-1 risk patients might in addition display symptomatic anemia, marked leukocytosis or constitutional symptoms such as drenching night sweats, fever or weight loss (cachexia). However, such symptoms are much more prevalent in intermediate-2 and high risk patients and they are usually not severe enough to warrant therapeutic intervention in lower-risk patients. Nevertheless, if treatment is indicated in low or intermediate-1 risk patients, it is reasonable to start with conventional drug therapy (see below) before rushing into treatment with experimental agents.

**How I manage intermediate-2 or high risk disease**
MF patients with high or intermediate-2 risk disease can be managed by conventional drug therapy, splenectomy, radiotherapy, allo-SCT or experimental drug therapy. With each one of these treatment modalities except allo-SCT, the primary goal is palliation of anemia, symptomatic splenomegaly, constitutional symptoms or disease complications from extramedullary hematopoiesis.

i. Conventional drug therapy

For the purposes of this review, I define “conventional drugs” as those that are FDA-approved and their utility in a specific disease-related complication has been published in a peer-reviewed medical journal. My approach in managing symptomatic anemia depends on presence or absence of associated splenomegaly. In the absence of splenomegaly, it is reasonable to try erythropoiesis stimulating agents (ESAs; e.g. darbepoietin 150-300 mcg weekly) based on a potential response rate of up to 56% (lasting for an average of 1 year) in patients that are not transfusion-dependent and show a hemoglobin level of <10 g/dL. It should be noted, however, that ESAs are unlikely to benefit patients who are transfusion-dependent or display a serum erythropoietin level of >125 U/L. Also, the use of ESAs is discouraged in the presence of more than mild splenomegaly (i.e. palpable spleen size >5 cm below the left costal margin), because of the danger of drug-induced exacerbation of splenomegaly.

In anemic patients who are either not good candidates for ESA therapy (see above) or in whom such therapy was unsuccessful, one has a choice of several potentially effective conventional drugs including corticosteroids (e.g. prednisone 0.5 mg/kg/day), androgens (e.g. fluoxymesterone 10 mg TID), danazol (600 mg/day), thalidomide (50 mg/day) ± prednisone (0.25 mg/day) or lenalidomide (10 mg/day) ± prednisone. Response rates and durations for each one of these treatment modalities are somewhat similar and estimated at 20% and 1 year, respectively. Thalidomide or lenalidomide should be avoided in women of childbearing age. Corticosteroid use should be avoided in the presence of diabetes or osteopenia; androgen or danazol use in the presence of
increased serum prostate specific antigen level or history of prostate cancer; thalidomide use in the presence of neuropathy; and lenalidomide use in the presence of moderate to severe neutropenia or thrombocytopenia.

I favor the use of lenalidomide in the presence of del(5q), and expect a response in both anemia and splenomegaly. In the absence of del(5q), the therapeutic benefit of lenalidomide, with or without prednisone, is too limited, and its myelosuppressive toxicity too high, to warrant its use as first-line therapy. Lenalidomide-treated patients should be closely monitored for the occurrence of severe myelosuppression and thrombosis; I use concomitant aspirin, if the platelet count is above 50 x 10^9/L, to minimize lenalidomide- or thalidomide-associated thrombosis. Thalidomide alone can produce approximately 20% response rates in MF-associated anemia, thrombocytopenia or splenomegaly. Furthermore, its use in combination with prednisone reduces the severity of its short-term side effects and might enhance its therapeutic activity. Unfortunately, with longer-term usage, a substantial proportion of patients develop thalidomide-associated peripheral neuropathy. Therefore, in the absence of del(5q), I usually try androgens or danazol before resorting to thalidomide; side effects of androgen therapy include hepatotoxicity and virilizing effects.

Hydroxyurea (starting dose 500 mg PO BID) is my first-line drug of choice for the treatment of MF-associated splenomegaly. In the presence of marked splenomegaly (palpable at >10 cm below the left costal margin), approximately 35% of patients were reported to achieve at least 25% reduction in spleen size–17% experienced 50% reduction in spleen size. Response rates were significantly lower (10%) in JAK2V617F-negative patients, compared to those with detectable JAK2V617F: 67% and 33% response rates in patients with mutant allele burdens of < or >50%, respectively. Spleen responses to hydroxyurea last for an average of one year and treatment side effects include myelosuppression, xerodermia and mucocutaneous ulcers. At present, participation in clinical trials
with JAK inhibitors (see below) is advised for patients with hydroxyurea-refractory splenomegaly or hepatomegaly. Otherwise, one can try intravenous cladribine (5 mg/m2 by 2-hour infusion daily x 5 days, and to be repeated monthly depending on toxicity and response),\textsuperscript{60} thalidomide\textsuperscript{52,57} or lenalidomide.\textsuperscript{53} Expected response rates for these latter agents range from 20% to 50%. Interferon (IFN)-\textgreek{a} is of limited value in the treatment of MF-associated splenomegaly.\textsuperscript{61}

\textit{ii. Splenectomy}

Splenectomy remains a viable treatment option for drug-refractory symptomatic splenomegaly in MF.\textsuperscript{62} In general, I consider splenectomy in the presence of marked splenomegaly (>10 cm palpable below the left costal margin) that is not responding to adequate doses of hydroxyurea and is associated with severe discomfort or pain, frequent red blood cell transfusions, severe thrombocytopenia, symptomatic portal hypertension or profound cachexia. The majority of MF patients that undergo splenectomy benefit from the procedure; more than half of transfusion-dependent patients become transfusion-independent and the majority also experience resolution of mechanical symptoms and improvement in constitutional symptoms, cachexia and platelet count if they were thrombocytopenic.\textsuperscript{63} The average duration of response is about a year.

The downside of splenectomy in MF includes a perioperative mortality rate of 5-10% and morbidity rate of approximately 25%.\textsuperscript{62,63} Abdominal vein thrombosis, operative site bleeding and infections are particularly prevalent during the post-operative period and close monitoring is critical for early diagnosis and treatment. In preparation for splenectomy, I usually place patients with platelet count of >200 x 10\textsuperscript{9}/L on hydroxyurea, in order to minimize the risk of post-operative extreme thrombocytosis and associated thrombosis.\textsuperscript{62} Post-operatively and once hemostasis is secured (about 5 to 8 days post-surgery), I usually put patients on therapeutic systemic anti-coagulation for about one month, in order to reduce the risk of post-operative splanchnic vein thrombosis. Post-splenectomy
thrombocytosis and left-shifted granulocytosis, including an increase in circulating blast percentage, are frequent and do not necessarily imply disease progression. These redistribution changes are often effectively managed by cytoreductive therapy. Also, approximately 20% of patients might experience progressive hepatomegaly and potentially useful drugs for such cases include hydroxyurea, cladribine or JAK inhibitors (see above in the immediately preceding section). Median survival after splenectomy has been reported to be about two years. In my opinion, leukemic transformation after splenectomy represents the natural progression of the disease rather than a treatment complication.\(^64\) Alternatives to splenectomy, in hydroxyurea-refractory splenomegaly, include participation in clinical trials, radiotherapy (see below) or transjugular intrahepatic portosystemic shunt (TIPS) in case of symptomatic portal hypertension (i.e. ascites, recurrent variceal bleed). Because of the lack of adequately sized relevant studies in MF, I do not recommend laparoscopic total or subtotal splenectomy or splenic artery embolization.

**iii. Radiotherapy**

In MF, radiotherapy is most useful in the setting of non-hepatosplenic EMH,\(^65\) pulmonary hypertension\(^66\) or lower or upper extremity pain.\(^67\) Non-hepatosplenic EMH might involve the vertebral column (spinal cord compression), lymph nodes (lymphadenopathy), pleura (pleural effusion) peritoneum (ascites), skin (cutaneous nodules) or other tissues and is effectively treated with low-dose radiotherapy (100-500 cGy in 5 to 10 fractions).\(^65\)

MF-associated pulmonary hypertension is suspected in the presence of clinical symptoms and signs including dyspnea/hypoxia on exertion and peripheral edema, increased systolic pulmonary artery pressure on echocardiography, and an abnormal pulmonary uptake during a technetium 99m sulphur colloid scintigraphy. It is important to rule out alternative causes such as thromboembolic, infectious or inflammatory lung processes (high resolution CT scanning helps in this regard). In the
absence of an alternative explanation for pulmonary hypertension, in a patient with MF, treatment with single-fraction (100 cGy) whole-lung irradiation is reasonable even if the technetium scan was negative.66

Single fraction of 100 to 400 cGy involved field radiotherapy has also been shown to benefit patients with MF-associated extremity pain.67 Finally, low-dose radiotherapy (100 cGy in 5-10 fractions) can be used in drug-refractory splenomegaly or hepatomegaly, and is capable of inducing a transient (3 to 6 months) reduction in organ size.68 Such treatment, however, is often associated with severe and sometimes protracted pancytopenia and is best reserved for those patients who are poor surgical candidates for splenectomy and are unable to participate in a clinical trial.68

**iv. Allogeneic stem cell transplant**

Aside from case reports involving lenalidomide use in patients with del(5q)-associated MF,56 allo-SCT is currently the only treatment option in MF that is capable of inducing complete hematologic, cytogenetic and molecular remissions.69 However, in considering allo-SCT as a treatment modality, one should be acutely aware of the risks involved. In the most recent study from the United Kingdom,70 51 PMF patients (24%, 33% and 43% with Dupriez low, intermediate and high risk disease)71 received mostly related conventional-intensity conditioning (CIC; ages 19-54 years) or reduced-intensity conditioning (RIC; ages 40-64 years) allo-SCT. Three-year overall survival (OS) was 44% for CIC and 31% for RIC transplant; the corresponding relapse rates were 15% and 46%, non-relapse mortality rates 41% and 32% and extensive chronic graft-versus-host disease (GVHD) rates 30% and 35%.70

In an earlier study from the Center for International Bone Marrow Transplant Research (CIBMTR) involving 289 patients with PMF (ages 18-73 years; 32%, 36% and 31% with Dupriez low, intermediate and high risk disease),72 treatment-related mortality (TRM) was 27% at 1 year and 35%
at 5 years. In the unrelated donor setting, TRM was 43% at 1 year, and 50% at 5 years. Five-year OS were 37% and 30% in related and unrelated donor settings, respectively. Outcome did not appear to be favorably affected by RIC where 3-year disease-free survival (DFS) was 39% and even lower (17%) in the unrelated donor setting. These results were similar to those of a multi-center study of 100 patients from Italy where RIC transplant did not affect the 3-year OS of 42% and TRM of 43%. A somewhat higher 5-year DFS (51%) was reported from another RIC allo-SCT study where relapse was predicted by high-risk disease and prior splenectomy. History of splenectomy did not affect outcome in the CIBMTR study.

Based on the above, I do not believe that the risk of allo-SCT is currently justified in DIPSS-plus low or intermediate-1 risk patients with MF (Figure 2). I am also not convinced that transplant-related mortality and morbidity in MF have been favorably altered by the use of RIC transplant where TRM, relapse and chronic GVHD rates remain uncomfortably high. Therefore, if allo-SCT is indicated because of high or intermediate-2 risk disease, I am inclined to favor the use of CIC transplant in younger patients (age <40-50 years), considering its association with a lower risk of relapse, compared to RIC transplant. It is reasonable to offer RIC transplant for older patients with high or intermediate-2 risk disease, especially if a matched related donor is available. There is currently no hard data to support the need for splenectomy before transplant. I prefer alternative means of reducing spleen size such as the use of cytoreductive therapy or JAK inhibitors (see below) although I recognize the unknown effect of the latter agents on post-transplant outcome.

v. Experimental drug therapy

Investigational drugs in MF include pomalidomide, JAK inhibitor ATP mimetics, histone deacetylase (HDAC) inhibitors (e.g. panobinostat, givinostat) and others (e.g. hypomethylating agents,
bevacizumab, plitidepsin). The most promising among these, so far, are pomalidomide and JAK inhibitors and each is further elaborated below.

**Pomalidomide**

*Is it better than thalidomide or lenalidomide?*

Pomalidomide is a thalidomide derivative classified with lenalidomide as an immunomodulatory drug (IMiD). In vitro, IMiDs antagonize angiogenesis and expression of TNF-α and IL-6 while they facilitate production of IL-2 and IFN-γ and enhance T cell and NK cell proliferation and activity; the precise mechanism of their action is not known but might include downregulation cytokine signalling. All three drugs (thalidomide, lenalidomide and pomalidomide) are active in both multiple myeloma and MF. In MF, thalidomide and lenalidomide, with or without prednisone, have comparable activity in alleviating anemia, splenomegaly and thrombocytopenia; response rates for each were in the vicinity of 20%. Treatment was complicated by peripheral neuropathy or severe myelosuppression in patients receiving thalidomide or lenalidomide, respectively. Therefore, there was room for improvement in both therapeutic activity and side effect profile.

In a phase-2 randomized study, approximately 25% of patients with anemia responded to pomalidomide alone (2 mg/day) or pomalidomide (0.5 or 2 mg/day) combined with prednisone. In a subsequent phase-2 study of single agent pomalidomide (0.5 mg/day), anemia response was documented only in the presence of JAK2V617F (24% vs. 0%) and predicted by the presence of pomalidomide-induced basophilia (38% vs. 6%) or absence of marked splenomegaly (38% vs. 11%). Platelet response was seen in 58% of patients with baseline platelet count of 50-100 x 10^9/L but the drug had limited activity in reducing spleen size. Unlike the case with thalidomide or lenalidomide,
drug-associated neuropathy or myelosuppression was infrequent. However, higher doses of pomalidomide (>2 mg/day) were myelosuppressive and not necessarily better in terms of efficacy.

In choosing between thalidomide, lenalidomide and pomalidomide, I prefer to use lenalidomide in the presence of del(5q), because of the possibility of obtaining hematologic and cytogenetic remissions.\textsuperscript{56} In the absence of del(5q), my decision hinges on the presence or absence of JAK2V617F or marked splenomegaly. My choice is pomalidomide for the JAK2V617F-positive patient without marked splenomegaly.\textsuperscript{76} Otherwise, it is reasonable to give thalidomide plus prednisone a try.\textsuperscript{51} Of note, I would not use any of these three drugs in the absence of symptomatic anemia.

### JAK inhibitors

**Value and limitations**

JAK2 inhibitor ATP mimetics that are currently in clinical trials include INCB018424, TG101348, CEP-701, CYT387, AZD1480, SB1518 and LY2784544 (\textit{clinicaltrials.gov}) (Table 4). Results of these studies so far suggest substantial differences among these drugs in their toxicity and efficacy profiles, some of which might be linked to their pharmacokinetic properties and variable \textit{in vitro} activity against other JAK and non-JAK kinase targets.

INCB018424 is a JAK1/JAK2 inhibitor. In a phase-1/2 study of 153 patients with MF,\textsuperscript{21} dose limiting toxicity (DLT) was thrombocytopenia and the maximum tolerated dose (MTD) was either 25 mg twice-daily or 100 mg once-daily. Adverse events included thrombocytopenia, anemia and a “cytokine rebound reaction” upon drug discontinuation. The latter is characterized by acute relapse of symptoms and splenomegaly, sometimes necessitating hospitalization. In a recent publication, 2 (1.3%) patients were reported to have experienced a Systemic Inflammatory Response Syndrome (SIRS) upon drug discontinuation. In order to prevent or decrease the intensity of cytokine rebound reaction, I avoid abrupt drug discontinuation and instead use a 2-week tapering schedule. In addition, I
sometimes use oral corticosteroid therapy (0.5 mg/kg/day), again in a tapering schedule, to help patients tolerate the event. Grade 3/4 thrombocytopenia or anemia in previously non-transfused patients occurred in 39% and 43% of patients receiving the drug at the recommended dose of 25 or 10 mg twice daily. At the lower dose level of 10 mg twice daily, the corresponding figures were 10% and 16%, but the response rate for splenomegaly (30%) was also lower at 10 mg twice daily compared to 25 mg twice daily (49%). Among all evaluable patients, 44% experienced ≥50% decrease in palpable spleen size. Improvement in constitutional symptoms (fatigue, pruritus, abdominal discomfort, early satiety, night sweats) and weight gain were seen in the majority of patients. Four (14%) of 28 transfusion-dependent patients became transfusion-independent. The drug’s effect on JAK2V617F allele burden or bone marrow pathology was negligible but a major reduction in proinflammatory cytokines (e.g. IL-1RA, IL-6, TNF-a, MIP-1b) was documented and coincided with improvement in constitutional symptoms.

TG101348 is a JAK2/FLT3 inhibitor. In a phase-1/2 study of 59 patients with MF, DLT was a reversible and asymptomatic increase in serum amylase and lipase and MTD was 680 mg/day. Grade 3/4 adverse events were all reversible and dose-dependent and included nausea (3%), vomiting (3%), diarrhea (10%), asymptomatic increases in serum lipase (27%), transaminases (27%) or creatinine (24%), thrombocytopenia (24%) and anemia (35%). After 6 and 12 months of treatment, 39% and 47% of patients, respectively, achieved a ≥50% decrease in palpable spleen size and the majority also experienced improvement in early satiety, fatigue, night sweats, cough or pruritus. Almost all patients with thrombocytosis and the majority with leukocytosis had drug-induced normalization of their counts. In general, responses were not affected by the presence or absence of JAK2V617F; however, a >50% decrease in mutant allele burden was seen in 39% patients with baseline JAK2V617F allele burden of >20%. Effect on bone marrow pathology or plasma cytokine levels were unremarkable.
CEP-701 is a JAK2/FLT-3 inhibitor. In a phase-2 study, 22 JAK2V617F-positive MF patients received the drug orally at 80mg twice-daily.\textsuperscript{77} A greater than 50% spleen reduction was achieved in four (22%) of 18 evaluable patients and two (25%) of 8 transfusion-dependent patients became transfusion independent.\textsuperscript{77} Grade 3/4 side effects included anemia (14%), thrombocytopenia (23%) and diarrhea (9%). The drug did not affect bone marrow fibrosis or JAK2V617F allele burden. Nineteen different plasma cytokines, including proinflammatory cytokines, were measured at baseline and post-treatment and showed no significant differences in their levels between responders and non-responders and between baseline and post-treatment samples.

Results from other JAK inhibitor clinical trials have not been published in full. However, glancing at the 2010 American Society of Hematology meeting abstracts reveals encouraging preliminary observations from a phase-1/2 study using another JAK1/JAK2 inhibitor, CYT387, where an impressive 40% response rate in anemia accompanied equally remarkable response rates in splenomegaly and constitutional symptoms.\textsuperscript{78} The drug was well tolerated with infrequent grade 3/4 adverse events that included thrombocytopenia in 22% of patients and anemia in only 3%. Information regarding other JAK inhibitors (AZD1480, SB1518 and LY2784544) was either less impressive or not available.

Based on the above discussion, three major points can be made regarding currently available JAK inhibitors and their value in MF. First, none of them were capable of inducing complete or partial remissions but they definitely have palliative value. Second, they are significantly different from each other in terms of both of therapeutic activity and side effect profile. The third point concerns their mechanism of action, which might involve downregulation of proinflammatory cytokines for INCB018424 and clonal myeloproliferation for TG101348. It is possible that the balance of their composite effect on these parameters governs the spectrum of their activity. In my opinion, the
therapeutically most promising JAK inhibitors at this point are CYT387, TG101348 and INCB018424 but longer follow up is needed to fully appreciate their safety profile.

**The future**

*Yes we can, but…*

None of the currently known mutations in *BCR-ABL1*-negative MPN exhibit *BCR-ABL1*-like disease specificity or pathogenetic significance. However, the remarkably high *JAK2* mutational frequency in these diseases justifies the flurry of current activity in preclinical and clinical evaluation of drugs that target JAK-STAT. I do not foresee an imatinib-CML like therapeutic success anytime soon because molecular pathogenesis in MF is complex and involves multiple mutations and aberrant pathways. It is possible that these putatively secondary changes are derived from a common mutant stem cell with a specific disease-causing mutation. It is also possible that MF is not one but many diseases with different molecular signatures. Either way, it is becoming more and more evident that JAK inhibitors, by themselves, may not constitute adequate therapy in MF, and have yet to show disease-modifying activity. Whether or not this will change by refining drug specificity to mutant as opposed to wild-type JAK remains to be seen. Nevertheless, JAK inhibitors have unquestionable palliative value and our challenge in this regard is to figure out which JAK inhibitor is appropriate for which disease, which patient, and at what point in the disease course. There is also room for improvement in terms of both therapeutic activity and side effect profile; for example, in addition to the now well-established value of certain JAK inhibitors (e.g. INCB018424, TG101348) in reducing spleen size and alleviating constitutional symptoms, preliminary results suggest that CYT387, a newer JAK1/2 inhibitor, also induces anemia response in a substantial proportion of patients.

Drug-induced complete remissions are possible in MF, as has been previously demonstrated for lenalidomide, in the presence of isolated del(5q). It is therefore critical to continue laboratory
investigations and new drug development with this goal in mind and not be content with what we have accomplished so far with JAK inhibitors. In this regard, PI3K/Akt/mTOR, Ras/MEK/ERK, STAT3/5 and HSP90 are alternative MPN-relevant drug targets with some encouraging preclinical and clinical data. There is also an obvious interest in combining these drugs with each other, or with conventional drugs such as hydroxyurea or ESAs. I am also intrigued by the significant anti-anemia activity of pomalidomide in JAK2V617F-positive MF. On the other hand, I have, thus far, not been impressed by the net value (benefit minus risk) of histone deacetylase inhibitors or hypomethylating agents. Finally, the recent demonstration of a correlation between JAK inhibitor therapy-induced downregulation of proinflammatory cytokines and clinical benefit in MF, combined with new information on specific plasma cytokines in PMF and their DIPSS-plus-independent prognostic relevance, support further evaluation of targeted anti-cytokine therapy.

**Author Contributions**

All content in the current paper were written by Ayalew Tefferi. He serves as as principle investigator or co-investigator on many clinical trials including those that are industry-sponsored. The latter include pomalidomide (Celgene), INCB018424 (Incyte), TG101348 (Targegene), CYT387 (YM Biosciences) and panobinostat (Novartis).
References


42. Tefferi A, Lasho TL, Huang J, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. Leukemia. 2008;22(4):756-761.


Table 1: World Health Organization classification of myeloid malignancies and operational subcategorization of myeloproliferative neoplasms

1) Acute myeloid leukemia and related precursor neoplasms

2) Myelodysplastic syndromes (MDS)

3) Myeloproliferative neoplasms (MPN)
   a) Classic MPN
      i) Chronic myelogenous leukemia, *BCR-ABL1* positive (CML)
      ii) Polycythemia vera (PV)
         (1) Chronic phase PV
         (2) Post-PV MF
         (3) Blast phase PV
      iii) Essential thrombocytemia (ET)
         (1) Chronic phase ET
         (2) Post-ET MF
         (3) Blast phase ET
      iv) Primary myelofibrosis (PMF)
         (1) Chronic phase PMF
         (2) Blast phase PMF
   b) Non-classic MPN
      i) Chronic neutrophilic leukemia
      ii) Chronic eosinophilic leukemia, not otherwise specified
      iii) Mastocytosis
      iv) Myeloproliferative neoplasm, unclassifiable (MPN-U)

4) MDS/MPN

5) Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRα*, *PDGFRβ*, or *FGFR1*

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*Genetic rearrangements involving platelet-derived growth factor receptor α/β (*PDGFRα/PDGFRβ*) or fibroblast growth factor receptor 1 (*FGFR1*).
Table 2: Somatic mutations described in classic myeloproliferative neoplasms including primary myelofibrosis, polycythemia vera and essential thrombocythemia.

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Chromosome location</th>
<th>Mutational frequency</th>
<th>Pathogenetic relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2 (Janus kinase 2)</td>
<td>9p24</td>
<td>PV ~ 96%, ET ~ 55%, PMF ~ 65%, BP-MPN ~ 50%</td>
<td>Contributes to abnormal myeloproliferation and progenitor cell growth factor hypersensitivity</td>
</tr>
<tr>
<td>JAK2V617F exon 14 mutation</td>
<td></td>
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<td></td>
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<tr>
<td>JAK2 exon 12 mutation</td>
<td>9p24</td>
<td>PV ~ 3%</td>
<td>Contributes to primarily erythroid myeloproliferation</td>
</tr>
<tr>
<td>MPL (Myeloproliferative leukemia virus oncogene)</td>
<td>1p34</td>
<td>ET ~ 3%, PMF ~ 10%, BP-MPN ~ 5%</td>
<td>Contributes to primarily megakaryocytic myeloproliferation</td>
</tr>
<tr>
<td>MPN-associated mutations involve exon 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TET2 (TET oncogene family member 2)</td>
<td>4q24</td>
<td>PV ~ 16%, ET ~ 5%, PMF ~ 17%, BP-MPN ~ 17%</td>
<td>May contribute to epigenetic dysregulation (TET proteins catalyze conversion of 5-methylcytosine to 5-hydroxymethylcytosine)</td>
</tr>
<tr>
<td>MPN-associated mutations occur across several of the gene’s 12 exons</td>
<td></td>
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<tr>
<td>ASXL1 (Additional Sex Combs-Like 1)</td>
<td>20q11.1</td>
<td>CP-MPN ~ rare, PMF ~ 13%, BP-MPN ~ 18%</td>
<td>Wild-type ASXL1 is needed for normal hematopoiesis and might be involved in transcriptional repression</td>
</tr>
<tr>
<td>Exon 12 mutations</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CBL (Casitas B-lineage lymphoma proto-oncogene)</td>
<td>11q23.3</td>
<td>PV ~ rare, ET ~ rare, MF ~ 6%</td>
<td>CBL is an E3 ubiquitin ligase that marks mutant kinases for degradation and transformi activity requires loss of wild type CBL</td>
</tr>
<tr>
<td>Exon 8/9 mutations</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IDH1/IDH2 (Isocitrate dehydrogenase)</td>
<td>2q33.3/15q26.1</td>
<td>PV ~ 2%, ET ~ 1%, PMF ~ 4%, BP-MPN ~ 20%</td>
<td>Induces formation of 2-hydroxyglutarate, a possible oncoprotein</td>
</tr>
<tr>
<td>Exon 4 mutations</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ikaros family zinc finger 1 (mostly deletions including intragenic)</td>
<td>7p12</td>
<td>CP-MPN ~ rare, BP-MPN ~ 19%</td>
<td>Transcription regulator and putative tumor suppressor</td>
</tr>
<tr>
<td>LNK (as in Links) a.k.a. SH2B3 (a membrane-bound adaptor protein)</td>
<td>12q24.12</td>
<td>PV ~ rare, ET ~ rare, PMF ~ rare, BP-MPN ~ 10%</td>
<td>Wild-type LNK is a negative regulator of JAK2 signaling</td>
</tr>
<tr>
<td>MPN-associated mutations were monoallelic and involved exon</td>
<td></td>
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<tr>
<td>EZH2 (enhancer of zeste homolog 2)</td>
<td>7q36.1</td>
<td>PV ~ 3%, PMF ~ 7%, MF ~ 13%</td>
<td>Wild-type EZH2 is part of a histone methyltransferase and might function both as a tumor suppressor (myeloid malignancies) and an oncogene (other tumors)</td>
</tr>
<tr>
<td>Both mono- and bi-allelic mutations occur in MPN, involving exons 10, 18 and 20, and are believed to be inactivating</td>
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</tbody>
</table>

Key: MPN, myeloproliferative neoplasms; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; MF includes both PMF and post-ET/PV myelofibrosis; BP-MPN, blast phase MPN; CP-MPN, chronic phase MPN
Table 3: Diagnostic criteria

**World Health Organization (WHO) diagnostic criteria for primary myelofibrosis (PMF) requires meeting all three major criteria and at least two minor criteria outlined below**\(^3\)

I. Major criteria
   a. Megakaryocyte proliferation including small to large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering accompanied by either reticulin and/or collagen fibrosis, or in the absence of reticulin fibrosis (i.e. prefibrotic PMF), the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (Figure 1c).
   b. Not meeting WHO criteria for chronic myelogenous leukemia, polycythemia vera, myelodysplastic syndromes, or other myeloid neoplasm
   c. Demonstration of JAK2V617F or other clonal marker or no evidence of reactive marrow fibrosis

II. Minor criteria
   a. Leukoerythroblastosis
   b. Increased serum lactate dehydrogenase
   c. Anemia
   d. Palpable splenomegaly

**International Working Group for Myeloproliferative Neoplasms Research and Treatment criteria for post-polycythemia vera/essential thrombocythemia (post-PV/ET) myelofibrosis requires meeting both major criteria and at least two minor criteria**\(^3\)

I. Major criteria
   a. Documentation of a previous diagnosis of PV or ET as defined by the WHO criteria
   b. Bone marrow fibrosis grade 2–3 (on 0–3 scale) or grade 3–4 (on 0–4 scale)*

II. Minor criteria
   a. A leukoerythroblastic peripheral blood picture (for both PV and ET)
   b. Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm (distance of the tip of the spleen from the left costal margin) or the appearance of a newly palpable splenomegaly (for both PV and ET)
   c. Development of ≥1 of three constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5°C) (for both PV and ET)
   d. Anemia or sustained loss of requirement for phlebotomy in the absence of cytoreductive therapy (for PV)
   e. Anemia and a ≥2 g/dL decrease from baseline hemoglobin level (for ET)
   f. Increased serum lactate dehydrogenase (for ET)

*Grade 2–3 according to the European classification: diffuse, often coarse fiber network with no evidence of collagenization (negative trichrome stain) or diffuse, coarse fiber network with areas of collagenization (positive trichrome stain). Grade 3–4 according to the standard classification: diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis or diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis.
Table 4: JAK2 inhibitors that have made it to clinical trials in myelofibrosis and not terminated as yet

<table>
<thead>
<tr>
<th>Anti-JAK2 ATP mimetic</th>
<th>Anti-JAK2 IC50 (JAK1/JAK3/TYK2 selectivity)</th>
<th>Non-JAK kinase targets</th>
<th>Clinical trials</th>
<th>Disease features shown to be favorably affected</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>INCBO18424&lt;sup&gt;22&lt;/sup&gt; (Phase 1/2 study)</td>
<td>5.7 nM (x1.0/x98/x9.3)</td>
<td>None of ~28 kinases evaluated</td>
<td>MF (n=153)&lt;sup&gt;21&lt;/sup&gt;</td>
<td>Splenomegaly Constitutional symptoms Pruritus Cachexia</td>
<td>Thrombocytopenia (DLT) Anemia “Acute relapse of symptoms and re-enlargement of spleen upon drug discontinuation” “Systemic inflammatory response syndrome (SIRS) upon drug discontinuation”</td>
</tr>
<tr>
<td>TG101348&lt;sup&gt;20&lt;/sup&gt; (Phase 1/2 study)</td>
<td>3 nM (x35/x332/x135)</td>
<td>FLT3 RET</td>
<td>MF (n=59)</td>
<td>Splenomegaly Constitutional symptoms Pruritus Leukocytosis Thrombocytosis JAK2V617F burden</td>
<td>Increased amylase/lipase (DLT) Anemia Thrombocytopenia Nausea/vomiting Diarrhea Increased transaminases</td>
</tr>
<tr>
<td>CEP-701&lt;sup&gt;27&lt;/sup&gt; (Lestaurtinib) (Phase 2 study)</td>
<td>1 nM (x?/x3/x?)</td>
<td>FLT3 TrkA</td>
<td>MF (n=22)</td>
<td>Splenomegaly Anemia Pruritus</td>
<td>Diarrhea Nausea/vomiting Anemia Thrombocytopenia</td>
</tr>
<tr>
<td>CYT387&lt;sup&gt;26&lt;/sup&gt; (Phase 1/2 study)</td>
<td>18 nM (x0.6/x8.6/x?)</td>
<td>JNK1 CDK2</td>
<td>MF (n=36)</td>
<td>Anemia Splenomegaly Constitutional symptoms Pruritus</td>
<td>Increased amylase/lipase (DLT) Headache (DLT) Thrombocytopenia Increased transaminases “First dose-effect characterized by transient hypotension and lightheadedness”</td>
</tr>
<tr>
<td>AZD1480&lt;sup&gt;29&lt;/sup&gt; (Phase 1/2 study)</td>
<td>0.26 nM (x5/x15/x?)</td>
<td>TrkA Aurora A FGFR1</td>
<td>MF</td>
<td>Results pending</td>
<td>Results pending</td>
</tr>
<tr>
<td>SB1518&lt;sup&gt;30&lt;/sup&gt; (Phase 1/2 study)</td>
<td>22 nM (x58/x24/x?)</td>
<td>FLT3</td>
<td>MF (n=31)</td>
<td>Splenomegaly</td>
<td>(DLT=GI symptoms) Diarrhea Nausea Thrombocytopenia</td>
</tr>
<tr>
<td>LY2784544 (Phase 1/2 study)</td>
<td>Scant literature</td>
<td>Scant literature</td>
<td>MF</td>
<td>Results pending</td>
<td>Results pending</td>
</tr>
</tbody>
</table>

Abbreviations: PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis; MF, myelofibrosis and includes PMF and post-PV/ET MF; DLT, dose-limiting toxicity; GI, gastrointestinal;
Legends

**Figure 1:** The Dynamic International Prognostic Scoring System (DIPSS)-plus prognostic model for primary myelofibrosis (PMF) uses 8 risk factors for inferior survival: age > 65 years, hemoglobin < 10 g/dL, leukocyte count > 25 x 10^9/L, circulating blasts ≥ 1%, presence of constitutional symptoms, presence of unfavorable karyotype, platelet count < 100 x 10^9/L and presence of red cell transfusion need.40

*Please note that a transfusion-dependent patient automatically has 2 risk factors because of transfusion need (one risk point) and hemoglobin <10 g/dL (one risk point).

**Constitutional symptoms constitute weight loss > 10% of baseline value in the year preceding diagnosis, unexplained fever or excessive sweats persisting for more than 1 month.38

***Unfavorable karyotype constitutes complex karyotype or sole or two abnormalities that include +8, -7/7q-, i(17q), inv(3), -5/5q-, 12p- or 11q23 rearrangement.41

**Figure 2:** Risk-adapted therapy in primary myelofibrosis. Key: DIPSS-plus, Dynamic International Prognostic Scoring System (DIPSS)-plus prognostic model for primary myelofibrosis;40 Int, intermediate; yrs, years; CIC, conventional intensity conditioning; RIC, reduced intensity conditioning; allo-SCT, allogeneic stem cell transplantation.

*Conventional drug therapy includes erythropoiesis stimulating agents, androgens, danazol, corticosteroids, thalidomide, lenalidomide, hydroxyurea and cladribine. Please see text regarding which agent is used when.
Figure 1:

**DIPSS-plus risk categories in primary myelofibrosis**

1. Low (no risk factors; median survival ~15.4 years)
2. Intermediate-1 (one risk factor; median survival ~6.5 years)
3. Intermediate-2 (2 or 3 risk factors; median survival ~2.9 years)
4. High (4 or more risk factors; median survival ~1.3 years)
Figure 2:

**Myelofibrosis treatment algorithm**

Yes  ➔  Is 5q- present? ➔  No

- **Lenalidomide** (in the presence of symptoms)

  - **DIPSS-plus prognostic category**
    - High risk or Int-2 risk
      - < 45 yrs.
        - Consider CIC allo-SCT
      - 45-66 yrs.
        - Consider RIC allo-SCT
      - > 65 yrs.
        - Investigational drug therapy
    - Int-1 risk
    - Low risk
      - Asymptomatic: Observation
      - Symptomatic: conventional drug therapy