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## Unraveling the genetic underpinnings of myeloproliferative neoplasms and understanding their effect on disease course and response to therapy: Proceedings from the 6<sup>th</sup> International Post-ASH Symposium

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### Abstract

Immediately after the annual scientific meeting of the American Society of Hematology (ASH), a select group of clinical and laboratory investigators in myeloproliferative neoplasms (MPN) is summoned to a post-ASH conference on chronic myeloid leukemia and the *BCR-ABL1*-negative MPN. The 6<sup>th</sup> such meeting occurred on 13<sup>th</sup>–14<sup>th</sup> December 2011, in La Jolla, California, USA, under the direction of its founder, Dr. Tariq Mughal. The current document is the first of two reports on this post-ASH event and summarizes the most recent preclinical and clinical advances in polycythemia vera, essential thrombocythemia and primary myelofibrosis.

### Keywords

ASXL1; EZH2; JAK2; myelofibrosis; myeloproliferative neoplasms; TET2; thrombocythemia

### Introduction

Since the discovery of the *JAK2*-V617F mutation in 2005, the myeloproliferative neoplasms (MPN) have been the objective of multiple gene discovery efforts. Exome and whole-genome sequencing in MPN are currently underway. In the meantime, use of array-based technologies and candidate gene sequencing have resulted in the identification of several novel somatic mutations (Table I). Prominent amongst these are alterations that affect epigenetic regulation of transcription, including mutations in *TET2* (1, 2) and *DNMT3A* (3, 4) as well as the recent discovery of mutations and copy-number loss in multiple members of the Polycomb Repressive Complex 2 (PRC2) (5–8). In the last year, much progress has been made in determining the functional implications of these mutations in both normal hematopoiesis and MPN pathogenesis. Currently, a great deal of effort has been placed on understanding the hierarchy of these somatic mutations, the order of mutation acquisition, and potential cooperativity of co-existing mutations in affecting the MPN phenotype. In parallel with increased understanding of the pathophysiology of MPN from genetic studies, recent therapeutic studies on the use of JAK inhibitors and pegylated-interferon alpha (PEG-IFN $\alpha$ ) have increased our pathogenetic insight in these diseases.

## Preclinical Advances in the Pathogenesis and Therapy of Myeloproliferative Neoplasms

### TET2 mutations

Somatic mutations in *TET2* are now well-established as occurring in patients with all myeloid malignancies including 7–16% of patients with polycythemia vera (PV), 4.4–11% with essential thrombocythemia (ET), and 7.7–17% of patients with primary myelofibrosis (PMF)(1, 2, 9) (Table I). When Delhommeau and colleagues first described *TET2* mutations in MPN, evidence for its importance in hematopoietic stem cell function was suggested by xenograft studies (1). The authors noted that injection of *JAK2V617F*-positive CD34 cells from MPN subjects with (n=2) or without *TET2* mutations (n=3) into NOD-SCID mice revealed a more efficient engraftment in the presence, as opposed to absence, of *TET2* mutations. *TET2* mutations could also be identified in the engrafted cells suggesting that the mutation was present at the stem/progenitor cell level. Moreover, the resulting hematopoiesis was skewed towards increased frequency of myeloid progenitors over lymphoid progenitors.

In the last year, additional knowledge regarding the biological function of TET2 in hematopoiesis has come from the study of mice with constitutive and conditional deletion of Tet2. Five different *Tet2* knockout mouse models were reported, all of which revealed the consistent finding that *Tet2* loss results in progressive expansion of the hematopoietic stem cell compartment and eventual myeloproliferation *in vivo*, including splenomegaly, monocytosis, and extramedullary hematopoiesis (10–13). In addition, *Tet2*<sup>+/-</sup> mice displayed increased stem cell self-renewal and extramedullary hematopoiesis, suggesting that *Tet2* haploinsufficiency contributes to hematopoietic transformation *in vivo* (12).

In parallel with the above-stated biological studies, the biochemical function of TET2 has been further clarified. TET2 is one of three members of Fe(II),  $\alpha$ -ketoglutarate dependent enzymes which serves to hydroxylate methylcytosine (14). Although not previously appreciated in mammals, 5-hydroxymethylcytosine (5-hmc) may block binding of methyl-binding proteins that are used to effect transcriptional silencing (15, 16). Studies in embryonic stem cells have demonstrated enrichment of 5-hmc marks in CpG dinucleotides near transcriptional start sites and within genes. 5-hmc may also lead to passive demethylation because Dnmt1 is unable to recognize 5hmc and methylation marks become lost through replication (17).

Recent studies have delineated a pathway where 5-hmc leads to active demethylation through DNA repair pathways. DNA deaminases including activation-induced cytidine deaminase (AID)/APoBEC are able to convert 5-hmc to 5-hydroxymethyl uracil (5-hmu). This is followed by a base excision repair mechanism that uses the enzyme thymine DNA glycosylase (TDG) or single-stranded monofunctional uracil DNA N-glycosylase SMUG1 (18, 19). Other studies have shown that TET enzymes may also affect conversion of 5-mc to 5-formylcytosine (5-fc) and 5-carboxylcytosine (5-cac); 5-cac can be directly recognized and repaired by TDG (20).

Loss of TET enzyme activity results in increased methylation and reduced 5-hmc levels. Patient samples with *TET2* mutations display lower global 5-hmc compared to their wild-type counterparts (21). Although defects in 5-hmc were also demonstrated in primary hematopoietic tissues from *Tet2* knockout mice, compared to controls in several of the *Tet2* knockout models, current work is focused on identifying targets of aberrant DNA methylation/hydroxymethylation, which may be important in regulating hematopoietic stem cell homeostasis.

Initial studies in MPN patients suggested the occurrence of *TET2*-mutant/*JAK2*-mutant and *TET2*-mutant/*JAK2*-wildtype clones, but not *TET2*-wildtype/*JAK2*-mutant clones, suggesting that *TET2* mutations occur as a “pre-*JAK2*” event (1). However, subsequent studies have noted the post-*JAK2*-V617F acquisition of *TET2* mutations, refuting a paradigm that mutations in *TET2* represent the earliest genetic aberration in MPN (22). Ongoing work examining cooperativity of *Jak2*-V617F mutations and *Tet2* loss *in vivo* will hopefully address the potential importance of the order of *JAK2/TET2* mutation acquisition on disease phenotype and HSC self-renewal in MPN (23).

### DNMT3a mutations

*DNMT3a* mutations have also been described in MPN (3, 4). Although these mutations are more frequent in AML and post-MPN AML, they also occur in chronic-phase MPN, including in 10–15% of PMF patients and 5–7% of PV patients. Until recently, data from murine studies suggested that *Dnmt3a* may be dispensable in HSCs (24). However, careful characterization of the hematopoietic stem cell biology from *Dnmt3a* knockout mice in serial transplantation experiments by Challen *et al.* has revealed that *Dnmt3a* loss results in a striking expansion of HSCs (25). Despite the dramatic effects of *Dnmt3a* loss on HSC number and frequency with serial transplantation, the effects of *Dnmt3a* loss on DNA methylation and gene expression are quite perplexing. From *in vivo* studies of genome-wide methylation status using HPLC-MS as well as bisulfite-sequencing of purified stem cell populations, there appears to be very little correlation between *Dnmt3a* loss and DNA methylation/gene expression at specific loci (25). Nonetheless, several genes which should be repressed for normal HSC differentiation were found to be consistently upregulated and hypomethylated with *Dnmt3a* loss including *Runx1* and *Gata3* (25). Although loss of *Dnmt3a in vivo* still appears from this work to be insufficient for transformation or disease phenotype, future work to address the combined effect of *Dnmt3a* loss with activating alterations in *Jak2* may be very enlightening.

### Mutations in Polycomb group proteins

In addition to mutations affecting enzymes regulating DNA methylation and demethylation, somatic mutations and copy-number alterations of genes regulating post-translational modification of histones have been recurrently found in patients with MPN and MPN/MDS overlap. These include recurrent mutations of *EZH2*, reported in 5–13% of patients with PMF (26, 27) and somatic mutations and copy-number alterations of other members of the Polycomb Repressive Complex 2 (PRC2).

Three independent groups have identified mutations in PRC2 core components *SUZ12* and *EED* or prominent deletions of PRC2 affiliated protein *JARID2* (6–8). Although these mutations are rare, occurring in 1–3% of patients with PMF, use of SNP array profiling by two groups has identified more frequent copy number deletion among all PRC2 members as well as deletions of PRC2 associated proteins *Jarid2* and *RBBP4* (7, 8). Recent clinical correlative work by Guglielmelli *et al.* revealed association of *EZH2* mutations with inferior overall survival and leukemia-free survival in PMF (28). Likewise, Puda *et al.* suggested that deletion of the genetic locus containing *Jarid2* (6p) might be associated with leukemic transformation (8).

In contrast to the accumulating genetic evidence for PRC2 loss-of-function alterations in MPN pathogenesis and leukemic transformation, comparatively less is known about the biological ramifications of these alterations. For instance, rigorous assessment of whether such mutations actually affect the abundance and/or distribution of H3K27me3 in chromatin has not yet been published, nor have the genetic targets of these alterations been clarified. Murine models with conditional deletion of *Ezh2* have been described (29). The myeloid

phenotype of Mx1-Cre/Ezh2 homozygous null was not fully described in this particular report. Further work to analyze the effects of PRC2 member deletion *in vivo*, alone and in combination with other oncogenic alleles, will hopefully allow for a deeper understanding of the contributions of these alterations to MPN and MDS pathogenesis.

### Resistance to JAK targeted therapies

Given the recent FDA approval of ruxolitinib (INCB018424) for myelofibrosis and the preclinical/clinical development of several additional JAK targeted therapeutics, a number of groups have recently investigated potential mechanisms of resistance to ATP-competitive JAK targeted therapies in *in vitro* mutagenesis paradigms. An initially published report by Hornakova *et al.* identified a number of spontaneously occurring mutations in the pseudokinase/kinase domains of JAK1, as well as JAK2, which result in both constitutive JAK-STAT activation as well as resistance to JAK targeted therapies (30). This study utilized a system of BaF3 cells transduced with a mutated IL-9R $\alpha$  to first identify spontaneously occurring mutations in JAK1 that result in autonomous cytokine-independent growth. This BaF3 cell line initially fails to grow in response to IL-9 but after prolonged culture eventually acquires responsiveness to IL-9 followed by acquisition of cytokine-independent growth. Prior characterization of these cells identified that spontaneously occurring mutations in endogenous JAK1 in addition to overexpression of JAK1 is responsible for this cytokine-independent transformation (31). Hornakova *et al.* then utilized this collection of BaF3 JAK1 mutant cells to examine their responsiveness to JAK inhibitor I and ruxolitinib. The authors thus identified 2 mutations in *JAK1* that resulted in constitutive JAK-STAT pathway activation and conferred resistance to both JAK inhibitor I and ruxolitinib: JAK1 F958 and JAK1 P960. Moreover, mutation at the homologous residue in JAK2 (JAK2 Y931) conferred resistance to both JAK inhibitors and resulted in cytokine-independent transformation of BaF3 cells (30).

Using a more systematic approach Deshpande *et al.* more recently identified additional point mutations in JAK2 which confer resistance to a wider variety of JAK inhibitors (32). These authors utilized a random mutagenesis screen in JAK2V617F cDNA followed by introduction into BaF3 cells expressing the erythropoietin receptor and identified 5 non-synonymous mutations in the drug-binding domain of JAK2 which resulted in resistance to ruxolitinib as well as to AZD1480, TG101348, lestaurtinib (CEP-701), and CYT-387. Interestingly one mutation (JAK2 Y931) matched that found by Hornakova *et al.* while the other 4 were unique (G935, R938, I960, and E985). The authors also attempted to find the homologous “gatekeeper” residue in JAK2 that matches the location of gatekeeper mutations in BCR-ABL1, KIT, and EGFR. Surprisingly, mutations at this residue (JAK2 M929) conferred resistance to only ruxolitinib (32). Despite these intriguing biochemical data, it is important to note that none of these genetic alterations have been identified in primary MPN patient samples to date and it is not yet clear how resistance to JAK inhibitors might be clinically defined. Recently, pre-clinical studies reported by Fiskus *et al.* demonstrated that co-treatment with heat shock protein (hsp) 90 inhibitor significantly increased JAK inhibitor mediated depletion of JAK/STAT signaling in primary MPN cells (33). Additionally, these studies showed that JAK inhibitor-resistant cultured human MPN cells, isolated under the *in vitro* selection pressure of JAK inhibitor, were collaterally highly sensitive to hsp90 inhibitor, thereby suggesting the *in vivo* testing of combined treatment with hsp90 inhibitor to overcome resistance to JAK inhibitors.

### HSC characterization in PMF

Recent advancements in the therapy of MPN patients have highlighted the need for increased understanding and targeting of the transformed hematopoietic stem cells underlying the MPN phenotype. While abnormalities in the mobilization, tracking, and

localization of HSCs have long been identified as a hallmark of PMF [51,52], very little functional characterization of the self-renewal and differentiation potential of HSCs from PMF patients has been performed previously. At the 2011 ASH meeting, Wang et al. presented an analysis of the engraftment and differentiation potential of HSCs from the peripheral blood and spleen of PMF patients [53]. The authors first identified that larger numbers of CD34+, CD34+CD38-, and CD34+CD90+ cells are present in the spleens of PMF patients compared with the peripheral blood. Secondly, the authors performed a series of xenografts of CD34+ cells from the spleen as well as peripheral blood of PMF patients into NOD/SCID/IL2R $\gamma$ null (nonobese diabetic severe combined immunodeficient interleukin-2 receptor gamma chain null) mice. Evaluation of the extent of human cell engraftment in the marrow of these mice following transplantation revealed that engraftment was superior in mice transplanted with splenic CD34+ cells compared with peripheral blood CD34+ cells. Moreover, splenic CD34+ cells appeared to be able to differentiate into myeloid as well as CD19+ and CD3+ cells whereas peripheral blood CD34+ cells appeared to be skewed towards myeloid differentiation. Collectively, these findings suggest that HSCs present in different compartments of PMF patients vary in their differentiation and engraftment potential. Further comparative analysis of the HSCs of PMF patients may reveal important findings regarding the hierarchy of hematopoietic stem progenitor cells in MPN patients as well as influences on the microenvironment and niche on HSC differentiation and homing.

### Role of inflammation in MPN pathogenesis

Inflammatory cytokines are markedly elevated in the sera of patients with MF and have been shown to be downregulated by some, but not other, JAK inhibitors (33, 34). Recent work by Tefferi *et al.* (35) and Pardanani *et al.* (36) have identified that serum cytokine profiling may additionally be utilized to predict outcome in patients with PMF and may also be useful in determining response to pomalidomide. In a seminal study, Tefferi *et al.* utilized a multiplex bead-based Luminex assay to profile the levels of a large number of serum cytokines in the sera of 127 patients with PMF (35). Careful clinical correlative work identified that elevations in 5 cytokines (IL-8, IL-2R, IL-12, IL-15, and IP-10) predicted for significantly worsened overall survival, independent of additionally clinically utilized variables in the revised Dynamic International Prognostic Scoring System. Additionally, leukemia-free survival was predicted by IL-8. An additional study from the same authors identified that elevations of several of these cytokines (IL-8 and IL-2R) are also inversely correlated with efficacy of pomalidomide therapy in treating anemia in PMF patients (36). Future work to integrate serum cytokine levels, clinical variables, and molecular genetic abnormalities in MPN patients may prove to be very enlightening in understanding MPN pathogenesis and disease course.

### Critical role of STAT5 in MPN pathogenesis

Increased laboratory and clinical investigations on the efficacy of JAK2 targeted therapies in patients with MPN has continued to show that JAK2 inhibitor therapies as currently administered are not able to eliminate the disease initiating clone in MPN patients (37) and in many cases may not effectively reduce allele burden (34, 38). This has led to ongoing interest in understanding the role of signaling events downstream of activated JAK2 in MPN.

*JAK2*<sup>V617F</sup> activates multiple signaling pathways including STAT5, STAT3, Erk/MAP kinase, and PI3 kinase/Akt. Recently, Yan *et al.* have demonstrated, using a *Jak2*<sup>V617F</sup> knock-in mouse model, that STAT5 is a critical mediator *in vivo* for development of the MPN phenotype (39). This was demonstrated genetically through crossing *Jak2*<sup>V617F</sup> knock-in mice to mice carrying homozygous conditional null mutations in the *Stat5ab* gene locus.



Deletion of *Stat5* completely abrogated the MPN phenotype including splenomegaly, erythrocytosis, and the increase in stem/progenitor compartment seen with Jak2V617F expression in the presence of STAT5. In contrast, use of a dominant-negative form of STAT3 or inhibitors of ERK or PI3K did not appear to affect the MPN phenotype induced by conditional *Jak2<sup>V617F</sup>* expression in mice (39). Similar findings were reported in a retroviral model of *Jak2<sup>V617F</sup>*-induced MPN, where deletion of *Stat5* completely reversed the polycythemia phenotype, but did not prevent the development of myelofibrosis (new ref: Walz et al Blood 2012, epub 2012-01-2012). These data suggest that signaling from JAK2 to STAT5 may be critical for hematopoietic transformation in MPN and targeting STAT5 may be therapeutically useful.

The phenotypic diversity displayed by JAK2-V617F remains an enigma. One prevailing hypothesis considers differential recruitment of signaling pathways downstream of JAK2 in patients with PV versus ET. Consistent with this hypothesis, Chen *et al.* recently showed that JAK2-mutated patients with ET preferentially activate STAT1, compared to patients with PV (40). This finding came from careful gene expression analysis in individual colonies from the blood of patients with PV and ET. The authors also showed that over-expression of STAT1 in normal CD34+ cells promoted megakaryocytic development and a phenotype akin to ET.

## Clinical Advances in the Prognostication and Therapy of Patients with Myeloproliferative Neoplasms

### New prognostic tools

In parallel with the interest in understanding the potential utility of genetic and serological factors to predict outcome in MPN patients, a number of advances in utilizing conventional clinical parameters to predict outcome were discussed at the ASH meeting and post-ASH symposium. The Dynamic International Prognostic Scoring System (DIPSS) score is currently the most widely utilized scoring system to predict outcome at time of diagnosis in PMF patients and includes age > 65 years, hemoglobin < 10g/dL, leukocytes greater than  $25 \times 10^9/L$ , circulating blasts  $\geq 1\%$ , and constitutional symptoms (41). In early 2011, the International Working Group for MPN Research and Treatment (IWG-MRT) developed a clinical prognostic algorithm incorporating karyotype, platelet count, and transfusion status to the DIPSS score to allow for outcome prediction at any time point in the clinical care of MF patients (42). More recently, Tefferi *et al.* performed a study of 884 PMF patients and identified that a smaller list of the DIPSS-plus score items can be utilized to more simply predict two-year predicted mortality in PMF patients (43). A greater than 80% 2-year mortality in PMF is predicted by the presence of a monosomal karyotype (defined as =2 autosomal monosomies or a single autosomal monosomy associated with at least one structural abnormality), inv(3) or inv(17)q abnormalities, or any 2 of the following: PB blast > 9%, WBC  $\geq 40 \times 10^9/L$ , or other unfavorable karyotype. The simplicity of this predictive algorithm may hopefully allow for improved risk stratification and management of PMF patients.

In addition to the studies above in PMF, the IWG-MRT has also recently performed a large international retrospective study of the natural history of PV including only patients diagnosed with PV using 2008 WHO diagnostic criteria. This study of 1,430 PV patients was presented at the 2011 ASH meeting and revealed that, with mature follow-up data, patients with PV appear to have a shortened lifespan compared with sex- and age-matched controls (44). Multivariate analysis identified advanced age, leukocytosis, venous thrombosis history and abnormal karyotype as significant ( $p < 0.01$ ) risk factors for overall survival in PV.

## Updates on JAK inhibitors

Since the initial publications of the phase I/II trial of ruxolitinib (33) and the phase I trial of TG101348 in patients with MF (45), knowledge about the role, schedule, and potential toxicities of a variety of JAK inhibitors continues to accumulate from clinical trials of these agents. At the 2011 ASH annual meeting, updates on 2 controlled trials of ruxolitinib in myelofibrosis (the COMFORT-I and -II trials) were presented (46, 47). COMFORT-I, a double-blind placebo controlled trial of ruxolitinib in MF, includes a total of 309 pts and clearly shows a benefit of ruxolitinib over placebo in reducing spleen volume and improving the Total Symptom Score at 24 weeks (47). Less clear are the effects of ruxolitinib on survival in MF from this study although this also appears potentially promising as 13 ruxolitinib-treated versus 24 placebo-treated patients have died so far (median follow-up of 52 and 51 weeks respectively), representing a hazard ratio (95% CI) of 0.499 (0.254, 0.98) ( $p=0.0395$ ). A more extended follow-up should address the effects of ruxolitinib on survival over placebo.

In addition to an update on COMFORT-I, preliminary results from COMFORT-II, a randomized open-label phase III trial of ruxolitinib compared with best available therapy in MF, was presented at the 2011 ASH meeting (46). Data from 219 patients randomized in a 2:1 fashion was presented and revealed a clear efficacy in ruxolitinib at reducing spleen size and improving symptoms and overall QoL, according to EORTC and FACT-LYMP scale. Currently, however, there is no evidence of a survival benefit; moreover, clear effects of ruxolitinib on *JAK2V617F* allele burden are not yet clear from these trials.

In an attempt to understand the effects of ruxolitinib on the natural history and survival of PMF while the COMFORT-I/II studies are ongoing, several investigators presented abstracts comparing the extended outcome of PMF patients from the phase I/II trial to historical controls (48–50). The resulting data regarding the effects of ruxolitinib on survival in PMF appear conflicting from these reports however. Nonetheless, it is clear from these reports that there are important potential adverse effects from ruxolitinib to be aware of such as worsening of cytopenias (including potentially irreversible thrombocytopenia) and serious withdrawal effects (rapid splenic enlargement and acute hemodynamic compensation) (48). Further clinical use of ruxolitinib and results from phase III trials will hopefully clarify the impact of this compound on the natural history of MF and the safety of this drug.

In addition to updated reports on ruxolitinib, clinical reports of several additional JAK inhibitors in MF were presented at the 2011 ASH meeting including a phase II study of pacritinib (SB1518) (51), a phase I/II study of SAR302503 (TG101348) (52), and a phase I/II study of CYT387 (53). Early data from these trials suggest that there may be a role for each of these JAK inhibitors in MF treatment as they may safely improve spleen size and constitutional symptoms at doses that induce minimal myelosuppression. Amongst these 3 reports, data from the phase I/II study of SAR302503 suggests that the compound may actually confer a durable decrease in *JAK2V617F* allele burden relative to the starting *JAK2V617F* allele burden (52). Data from the phase I/II study of CYT387 continues to suggest a unique role for this drug in MF as it appears to result in significant improvements in anemia (53). This observation is of considerable interest and the potential underlying molecular mechanism speculative. The most common grade 3–4 toxicity of CYT387 is thrombocytopenia (occurring in 16% of subjects). Further follow-up of all of these studies will continue to inform our understanding of the potential role, specific adverse effects, and dosing schedules of the increasing variety of JAK inhibitor therapies.



## Interferon-alpha in the treatment of MPN patients

In addition to the burgeoning use of JAK2 targeted therapies in MPN patients, a number of clinical studies updating results on the use of pegylated interferon alpha-2a (peg-IFN- $\alpha$ 2a) were presented at the ASH 2011 meeting. Updated data on a phase II trial of peg-IFN- $\alpha$ 2a in 40 patients with high-risk PV and ET from Turlure *et al.* continue to reveal the efficacy of peg-IFN- $\alpha$ 2a in producing durable complete hematologic and molecular remissions in these patients (54). In this study, 94% of patients remained with a sustained hematological response, including 82% with complete remission, after 6.4 years median follow-up. Moreover, 29% of patients were able to stop peg-IFN $\alpha$ -2a and sustained a hematological response without further cytoreductive therapy after a median observation time of more than 28 months (with one patient up to 64 months). 28% of the patients in this study achieved a complete molecular response, as measured by *JAK2*-V617F allele burden in granulocytes.

Kiladjian *et al.* studied several patients on PEG-IFN- $\alpha$ 2a (55) and found persistence of *TET2* mutations despite eradication of *JAK2* mutations in patients with *JAK2/TET2* mutations. Quintas-Cardama *et al.* similarly performed molecular studies in high-risk PV and ET patients undergoing treatment with peg-IFN- $\alpha$ 2a (56) and demonstrated that patients who failed to achieve a complete molecular remission were statistically more likely to have a mutation outside of *JAK2* (e.g. *TET2*, *DNMT3A*) during the course of therapy. The findings from the 2 studies above suggest a possible influence of additional mutations outside of *JAK2* on resistance to PEG-IFN- $\alpha$ -2a therapy. It will be interesting to learn if mutations in genes other than *JAK2* are also differentially affected by JAK inhibitor therapy.

## Pomalidomide, HDAC inhibitors, and allogeneic stem cell transplantation

Currently a phase III trial of the investigational immunomodulatory drug pomalidomide is underway as a single agent in the therapy of patients with MF based on data from phase II randomized trials revealing that the pomalidomide at 0.5mg to 2mg per day is active in MF and effective at improving anemia (57). Longer-term follow-up data from patients on phase I and phase II trials were presented at the 2011 ASH meeting and revealed that responses to pomalidomide most commonly occur at a median of 2.3 months after the start of therapy and are durable for a median of 16.5 months (range 2–40 months) (58). An additional phase II trial using a low (0.5mg) continuous daily oral dose of pomalidomide revealed that although a portion of transfusion-dependent patients did achieve transfusion independence with this dosing strategy at a median followup of more than 1 year, no patient experienced a sustained increase in hemoglobin of 2g/dL from baseline (59).

In addition to ongoing studies of JAK inhibitors, pomalidomide, and PEG-IFN- $\alpha$ -2a in therapy of patients with MPNs, a number of studies continue to determine the efficacy of a variety of histone deacetylase inhibitors (HDACi) in MPN patients. Mascarenhas *et al.* presented the final results of a phase I study of the pan-HDACi LBH589 (panobinostat) in MF patients at the 2011 ASH meeting (60). 18 patients were enrolled at 3 different dose levels including 5 patients who have received > 6 months of therapy. Interestingly, several patients experienced near CR results with extended therapy. DeAngelo *et al.* also presented findings demonstrating that treatment with panobinostat reduces in vivo *JAK2* levels, *JAK2*V617F allele burden, JAK-STAT signaling, and inflammatory cytokine levels in patients treated on a separate clinical trial of panobinostat (61). Based on these trials, a phase II trial of LBH589 is underway with 25mg po TIW as the recommended dose. In addition to the study of LBH589 in MF, Rambaldi *et al.* presented the results of a phase II study of the HDACi givinostat plus hydroxyurea (HU) for HU-resistant PV patients (61). This study revealed that the combination of givinostat plus HU is well tolerated and ~50% of patients with HU-resistant disease achieved a PR or CR with combination of HU plus givinostat.

Finally, in addition to the above studies on pharmaceutical agents in the therapy of MPN patients, the results of a recently completed prospective study of allogeneic stem cell transplantation in MF were presented at the 2011 ASH meeting. This study, conducted by the MPD-Research Consortium, utilized a reduced-intensity conditioning regimen consisting of fludarabine and melphalan (62). Gradt-versus host disease prophylaxis was with tacrolimus and methotrexate. In addition, patients undergoing unrelated donor transplantation or those with mismatched donor also received ATG. The initial results from this study suggest that this conditioning regimen followed by transplant from a sibling donor was very effective while MF patients undergoing unrelated stem cell transplants had a high-rate of transplant-related mortality (53% of such treated patients died from causes related to transplant).

## Conclusions

*BCR-ABL1*-negative MPN are characterized by recurrent mutations that activate the JAK-STAT pathway (mutations in *JAK2*, *MPL*, and *LNK*), dysregulate DNA cytosine methylation/demethylation (*TET2*, *DNMT3A*), or alter post-translational modifications of histones (*EZH2*, *SUZ12*, *EED*, *JARID2*). The systematic evaluation of these genetic alterations in genetically-engineered mouse models has continued to inform our understanding of MPN pathogenesis and HSC biology. In addition, these mutant molecules may prove useful as drug targets or biomarkers for either predicting prognosis or monitoring treatment response. More recent data regarding cytokines and parameters that quantify host immune response suggest their additional value in disease prognostication.

Currently an increasing range of therapies is being investigated in MPN patients including multiple early phase and phase III trials of a variety of JAK inhibitors, pomalidomide, HDACi, and allogeneic stem cell transplantation with reduced-intensity conditioning (Table II). Data from the ongoing trials of ruxolitinib, pacritinib, SAR302503, and CYT387 will be critical in determining the safety of these drugs, their efficacy compared with best alternative conventional therapies, and the unique clinical properties of each compound.

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Frequency of somatic mutations and genetically disordered pathways in patients with classic myeloproliferative neoplasms

Table I

General pathway/Complex	Gene	Polycythemia vera	Essential thrombocytosis	Primary myelofibrosis	Comment
JAK-STAT signaling	<i>JAK2</i>	81–99%	41–72%	39–57%	Expression of activated mutant forms of JAK2 or MPL is sufficient for MPN disease phenotype <i>in vivo</i> but it is not clear whether inhibition of JAK2/MPL signaling is sufficient for cure of MPN disease.
	<i>MPL</i>	Not reported	3–5%	8–10%	
	<i>LNK</i>	Present	3–6%	3–6%	
	<i>CBL</i>	Rare	Rare	6%	
DNA methylation/demethylation	<i>TET2</i>	7–16%	4.4–11%	7.7–17%	Deletion of <i>Cbl</i> <i>in vivo</i> results in expansion of HSC pool, splenomegaly, and enhanced sensitivity to growth factors. Deletion of <i>Tet2</i> or <i>Dnmt3a</i> has been shown to result in hematopoietic stem cell expansion <i>in vivo</i> .
	<i>DNMT3A</i>	5–7%	Reported	10–15%	
	<i>EZH2</i>	3–5%	Reported	5.9–13%	
Polycomb Repressive Complex 2	<i>SUZ12</i>	Not reported	Not reported	Mutations associated with shortened overall survival in PMF, independent of IPSS score. Deletion of <i>SUZ12</i> , <i>EED</i> , <i>Jarid</i> loci is recurrent in MPN patients and appears to be associated with leukemic transformation.	
	<i>EED</i>				
	<i>Jarid</i>		Not reported		
Not clarified	<i>ASXL1</i>	2–5%	5–6%	13–23%	Mutations in <i>ASXL1</i> recently shown to be adverse prognosticator in MDS and AML but clinical importance in MPN patients not yet clear. Biological role of <i>ASXL1</i> in hematopoiesis is also not yet known. Gain of function mutations in <i>IDH1/2</i> result in DNA hypermethylation and alterations in a number of histone methylation modifications due to effect on TET family of enzymes and Jumonji family of histone lysine demethylases.
	<i>IDH1/2</i>	Rare	Rare	4%	

**Table II**

Current Therapies Under Investigation for the Treatment of the Myeloproliferative Neoplasms (MPNs)  
Presented at the 2011 American Society of Hematology Annual Meeting and/or Published in 2011

Class of therapy <sup>a</sup>	Compound	Study <sup>b</sup>	Trial design <sup>c</sup>	Results/comment <sup>d</sup>
JAK inhibitors	Ruxolitinib	COMFORT-I, double-blind placebo controlled trial in MF patients [13].	Data from 309 patients reported, benefit over placebo in reducing spleen volume and improving Total Symptom Score at 24 weeks.	Potential beneficial effect on survival compared with placebo in MF but more extended follow-up will be needed. Conflicting reports on survival and safety of ruxolitinib from retrospective follow-up of phase I/II trial.
		COMFORT-II, randomized open-label phase III trial compared with best available therapy in MF patients [14].	Data from 219 patients reported, 2:1 randomization, benefit over placebo in reducing spleen volume and improving quality of life metrics.	Data on effect of ruxolitinib at reducing JAK2V617F allele burden are not clear from initial results of COMORT-I and -II.
	SAR302503	Phase I for MF patients [15].	28 patients treated in dose-escalation cohort and 31 at maximum tolerated dose (680 mg daily).	Significant decrease in spleen and JAK2V617F allele burden with minimal toxicities.
	CYT387	Phase I/II for MF patients [16].	Multicenter study with single and twice-daily doses investigated.	Substantial activity in reducing spleen size, improving constitutional symptoms, and improving anemia. Thrombocytopenia most significant side effect (16% subjects).
	Pacritinib	Phase II trial in MF patients [17].	Data from 34 patients reported. Drug administered on a once daily dose.	Once daily dose has minimal myelosuppression and alleviates splenomegaly and constitutional symptoms. GI toxicity appears to be main side effect thus far.
HDACi	Panobinostat	Phase I trial in MF patients [18].	Eighteen patients at 3 different dose levels, several patients have experienced near CR.	Phase II trial in MF is now underway with 25 mg po TIW.
	Givinostat + hydroxyurea	Phase II for hydroxyurea-resistant PV patients [19].	Forty-four patients with JAK2V617 PV enrolled.	~50% patients achieved a PR or CR based on ELN guidelines.
PEG-IFN- $\alpha$ 2a		Phase II trial for patients with high-risk PV [20].	Forty patients treated with median follow of 77.4 months.	94% had a sustained hematological response, including 82% with CR, after 6.4 years median follow-up. 28% achieved complete molecular response. 29% were able to stop peg-IFN $\alpha$ -2a and sustained hematological response without further therapy after a median time of >28 months.
Pomalidomide		Phase I/II trial in MF patients [21].	Doses ranging from 0.5 to 2 mg in 83 patients.	Improvement in anemia occurred at median of 2.3 months and durable for median of 16.5 months.
		Phase II trial in MF patients [22].	0.5 mg dose studied in 28 patients.	A proportion of patients achieved transfusion-independence but none with sustained increase in hemoglobin >2 g/dL.
Allogeneic hematopoietic stem cell transplantation		Prospective study of allogeneic transplant with reduced intensity	Thirty-two patients received allogeneic transplant from related donor and 34 from an unrelated donor.	Transplantation from a sibling donor was very effective while transplants from unrelated donors had a very high-rate (53%) of transplant-related mortality.

Class of therapy <sup>a</sup>	Compound	Study <sup>b</sup>	Trial design <sup>c</sup>	Results/comment <sup>d</sup>
		conditioning in MF patients [23].		

<sup>a</sup>HDACi- histone deacetylase inhibitors.

<sup>b</sup>ET—essential thrombocytosis; MF—myelofibrosis; PV—polycythemia vera.

<sup>c</sup>CR—complete remission; PR-partial remission.

<sup>d</sup>ELN: European Leukemia Net; GI: gastrointestinal.