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MEETING REPORT

Mutations with epigenetic effects in myeloproliferative neoplasms and recent progress in treatment: Proceedings from the 5th International Post-ASH Symposium

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Immediately following the 2010 annual American Society of Hematology (ASH) meeting, the 5th International Post-ASH Symposium on Chronic Myelogenous Leukemia and *BCR-ABL1*-Negative Myeloproliferative Neoplasms (MPNs) took place on 7–8 December 2010 in Orlando, Florida, USA. During this meeting, the most recent advances in laboratory research and clinical practice, including those that were presented at the 2010 ASH meeting, were discussed among recognized authorities in the field. The current paper summarizes the proceedings of this meeting in *BCR-ABL1*-negative MPN. We provide a detailed overview of new mutations with putative epigenetic effects (TET oncogene family member 2 (*TET2*), additional sex comb-like 1 (*ASXL1*), isocitrate dehydrogenase (*IDH*) and enhancer of zeste homolog 2 (*EZH2*)) and an update on treatment with Janus kinase (JAK) inhibitors, pomalidomide, everolimus, interferon-α, midostaurin and cladribine. In addition, the new 'Dynamic International Prognostic Scoring System (DIPSS)-plus' prognostic model for primary myelofibrosis (PMF) and the clinical relevance of distinguishing essential thrombocythemia from prefibrotic PMF are discussed.

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Keywords: myeloproliferative; myelofibrosis; polycythemia; thrombocythemia; mastocytosis

Introduction

The World Health Organization (WHO) classification system for myeloid malignancies uses morphology, in combination with cytochemical, immunophenotypic, cytogenetic and molecular data, to classify myeloid malignancies into five major categories: acute myeloid leukemia (AML), myelodysplastic syndromes (MDSs), myeloproliferative neoplasms (MPNs), MDS/MPN, and *PDGFR*- or *FGFR1*-rearranged myeloid/lymphoid neoplasms associated with eosinophilia.[1] The WHO MPN category includes chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), mastocytosis, chronic eosinophilic leukemia—not otherwise specified, chronic neutrophilic leukemia and MPN, unclassifiable.[2] CML, PV, ET and PMF are referred to as 'classic' MPN because they were included in the original description of 'myeloproliferative disorders' by William Dameshek.[3]

Early seminal work by Fialkow and colleagues[4–7] had established MPN as clonal stem-cell diseases, with lymphoid lineage involvement in some instances.[8,9] More recent studies have confirmed these observations[10–12] and further suggest the possibility of independently emerging multiple abnormal clones, which might lead to oligoclonal rather than monoclonal myeloproliferation.[13] Although there is evidence for genetic predisposition in MPN,[14–19] the link is not strong enough to warrant family screening, and Janus kinase 2 (*JAK2*) 46/1 haplotype analysis studies have shown similar frequency between familial and sporadic cases of MPN.[20]

To date, the disease-initiating event(s) in *BCR-ABL1*-negative MPN has not been identified. However, beginning in 2005, a number of stem-cell-derived[21–26] mutations involving *JAK2* (exon 14 (refs 27–30) and exon 12 (ref. 31)), *MPL* (exon 10 (refs 32,33)), *TET2* (ref. 25), additional sex comb-like 1 (*ASXL1*; exon 12 (ref. 26)), *CBL* (exons 8 and 9 (ref. 34)), isocitrate dehydrogenase 1 (*IDH1*; exon 4 (refs 35–38)), *IDH2* (exon 4 (refs 35–37,39)), IKAROS family zinc finger 1 (*IKZF1*) (ref. 40) and enhancer of zeste homolog 2 (*EZH2* (refs 39,41)) have been described in chronic or blast-phase MPN. All of these mutations are currently believed to represent secondary events and are known to coexist. In this regard, any claim of mutual exclusivity is undermined by the very low mutational frequency displayed by the majority of the mutations. Activating *JAK2* and *MPL* mutations and *LNK* loss-of-function result in constitutive JAK–signal transduction and activator of transcription (STAT) activation and induce MPN-like disease in mice.[27,32,42,43] *TET2*, *ASXL1*, *IDH* and *EZH2* mutations might contribute to epigenetic dysregulation of transcription and are further discussed in the current review (Table 1). However, it should be noted that some mutations might possess more than one mechanism of action, for example, *JAK2*V617F results in dysregulation of kinase signaling but might also have an epigenetic effect.[44,45] The current review will also highlight recent clinical advances in MPN including the development of JAK–STAT-targeted therapy and application of new prognostic models.

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Table 1 Epigenetically implicated mutations in myeloid malignancies

Mutations	Chromosome location	*Mutational frequency*	*Pathogenetic relevance*
TET2 mutations involve several exons[25,146]	4q24	PV ~16%[146] ET ~5%[146] PMF ~17%[146] BP-MPN ~17%[146] AML ~20%[57] MDS ~26%[58] CMML ~51%[56] SM ~29%[52] RARS-T ~26%[59]	TET proteins catalyze conversion of 5mC to 5hmC, which favors demethylated DNA. Both TET1[66] and TET2[67] display this catalytic activity. *IDH* and *TET2* mutations might share a common pathogenetic effect, which might include abnormal DNA hypermethylation and impaired myelopoiesis.
ASXL1 exon 12 mutations[26]	20q11.1	ET ~3%[72] PMF ~13%[39] BP-MPN ~18%[39] AML ~11%[76] MDS ~11%[26] CMML ~43%[26]	Wild-type ASXL1 is needed for normal hematopoiesis[69] and might be involved in coactivation of transcription factors and transcriptional repression.[70,71]
IDH1/IDH2 exon 4 mutations[35]	2q33.3/ 15q26.1	PV ~2%[35] ET~1%[35] PMF ~4%[35] BP-MPN ~20%[35] AML ~14%[87] MDS ~5%[55]	*IDH* mutations induce loss of activity for the conversion of isocitrate to 2-KG and gain-of-function in the conversion of 2-KG to 2-HG.[81,82] 2-HG might be the mediator of impaired TET2 function in cells with mutant *IDH* expression.[68]
EZH2 mutations involve several exons[41]	7q36.1	PV ~3%[41] PMF ~7%[39] MDS ~6%[41,97] CMML ~13%[41] aCML ~13%[41] HES/CEL ~3%[41]	Wild-type EZH2 is part of a histone methyltransferase (polycomb-repressive complex 2 associated with H3 Lys-27 trimethylation). MPN-associated *EZH2* mutations might have a tumor-suppressor activity,[41] which contrasts with the gain-of-function activity for lymphoma-associated *EZH2* mutations.[93]

Abbreviations: aCML, atypical chronic myeloid leukemia, *BCR-ABL1*-negative; AML, acute myeloid leukemia; ASXL1, additional sex comb-like 1; BP-MPN, blast phase myeloproliferative neoplasm; CMML, chronic myelomonocytic leukemia; CP-MPN, chronic phase MPN; ET, essential thrombocythemia; EZH2, enhancer of zeste homolog 2; HES/CEL, hypereosinophilic syndrome/chronic eosinophilic leukemia; IDH, isocitrate dehydrogenase; MDS, myelodysplastic syndrome; PMF, primary myelofibrosis; PV, polycythemia vera; RARS-T, refractory anemia with ring sideroblasts; SM, systemic mastocytosis; TET2, TET oncogene family member 2; 2-HG, 2-hydroxyglutarate; 2-KG, 2-ketoglutarate; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine.
MF includes both PMF and post-ET/PV myelofibrosis.

New mutations in MPNs with putative epigenetic effect

TET2 mutations

TET2 (TET oncogene family member 2) maps to chromosome 4q24. *TET2* mutations were first discovered in MPN by Bernard's team from France and occur across several of the gene's 12 exons.[25] Subsequently, Mayo Clinic (Rochester, MN, USA) investigators in collaboration with colleagues from Memorial Sloan-Kettering (New York) and Dana-Farber (Boston) Cancer Centers described *TET2* mutational frequencies of ~16% in PV, 5% in ET, 17% in PMF and 17% in blast-phase MPN.[46] Out of total 32 *TET2* mutations in the latter study,[46] 19 were frameshift, 10 nonsense and 3 missense, and involved mostly exons 4 or 12. *TET2* mutational frequency was ~23% in patients 60 years of age or older versus 4% in younger patients, and this accounted for the difference in mutational frequency between *JAK2*V617F-positive (17%) and -negative (7%) cases; *JAK2*V617F is associated with older age at diagnosis.[47] In this particular study,[46] the presence of mutant *TET2* was not prognostically relevant. *TET2* mutation acquisition can antedate or follow *JAK2*V617F, and can also coexist with various cytogenetic abnormalities[48,49] or mutations in *MPL, RARA, KIT, ASXL1* or *IDH*.[38,39,50–55]

TET2 mutations also occur in other myeloid malignancies, including mastocytosis (~29%),[52] chronic myelomonocytic leukemia (CMML; ~51%),[56] AML (~20%),[57] MDS (26%),[58] refractory anemia with ring sideroblasts (~26%)[59] and idic(X)(q13)-positive myeloid malignancies.[60] In a recent study,[61] *TET2* mutations were reported in 39 (12%) of 320 MDS cases and 16 (46%) of 35 CMML cases.[61] As was the case in MPN,[46] older age was associated with a higher incidence of *TET2* mutations, which did not otherwise affect prognosis in either MDS or CMML.[61] These results are different from another MDS study where *TET2* mutational frequency was reported at 23% and the mutation had an independent favorable impact on survival.[62] Discrepant results on the prognostic effect of mutant *TET2* have also been reported in AML, secondary acute myeloid leukemia (sAML) and CMML.[38,46,50,56,57,61,63] At the American Society of Hematology (ASH) 2010, a study on the prognostic impact of *TET2* mutations in 783 uniformly treated young AML patients was presented and showed no effect on survival, including in subgroups with normal karyotype or $NPM1^{+}FLT3/ITD^{-}$ molecular profile.[64] In another ASH abstract, however, the presence of mutant *TET2* was associated with poor prognosis in the context of favorable but not intermediate-risk cytogenetically normal AML.[65]

TET proteins belong to a family of α-oxaloglutarate-dependent enzymes and catalyze conversion of 5-methylcytosine to 5-hydroxymethylcytosine, which favors demethylated DNA. Both TET1[66] and TET2[67] display this catalytic activity, and bone marrow-derived DNA from *TET2*-mutated patients display low levels of 5-hydroxymethylcytosine.[67] In a recent study in AML,[68] *TET2* and *IDH* mutations were mutually exclusive but shared similar epigenetic defects, including extensive DNA promoter hypermethylation and hypermethylation of a specific set of gene

promoters (that is, displayed a similarly specific epigenetic signature). Furthermore, *in vitro* induction of mutant but not wild-type *IDH* expression in cells impaired TET2 catalytic activity, presumably because of generation of 2-hydroxyglutarate, which can interfere with TET2 function.[68] Similarly, depletion of TET in mouse hematopoietic precursors skewed their differentiation toward monocyte/macrophage lineages.[67] Taken together, these data suggest a common pathogenetic effect for *IDH* and *TET2* mutations, which might include abnormal DNA hypermethylation and impaired myelopoiesis. On the other hand, it is difficult to explain the inconsistent finding from another study where low 5-hydroxymethylcytosine level was associated with DNA hypomethylation.[67]

ASXL1 mutations

ASXL1 maps to chromosome 20q11.1. *ASXL1* mutations involve exon 12 and truncate the pleckstrin homology domain of ASXL1. Wild-type ASXL1 is needed for normal hematopoiesis[69] and might be involved in coactivation of transcription factors and transcriptional repression.[70,71] A recent study showed that *ASXL1* is expressed in most hematopoietic cells, and *ASXL1* knockout mice did not show MDS phenotype or stem-cell defects, although they displayed impaired differentiation of lymphoid and myeloid progenitors.[69]

ASXL1 mutations were first described by Gelsi-Boyer *et al.*[26] who studied 40 cases of MDS or AML and found *ASXL1* exon 12 mutations in 4 (11%) of 35 MDS cases and in 17 (43%) of 39 CMML cases. The same group of investigators subsequently studied 64 patients with chronic or blast-phase MPN and detected heterozygous frameshift mutations of *ASXL1* in 5 (~8%) cases including 1 (3%) of 35 ET, 3 (30%) of 10 PMF and 1 post-ET AML.[72] In this particular study, *ASXL1* mutations were exclusive of *JAK2*V617F, whereas one PMF case was also mutated for *TET2*. In yet another study,[73] the same authors studied 63 AML cases including 46 with normal karyotype; they reported 12 (19%) cases with *ASXL1* mutations that were mutually exclusive of *NPM1* mutations. *ASXL1* mutational frequency in another study of 63 post-MPN cases was also 19%.[38]

Among 300 patients with MDS, AML or CMML, *ASXL1* mutations were reported in 62 patients, including 5 (~6%) of 79 patients with refractory anemia, 17 (~31%) of 55 patients with refractory anemia with excess blasts and 17 (25%) of 67 patients with AML.[74] The same group of investigators subsequently reported 6 *ASXL1* mutations among 41 cases with chronic or blast-phase CML.[75] In a more recent study of 501 adults with *de novo* AML, *ASXL1* mutations were detected in 54 patients (~11%) and were associated with presence of isolated trisomy 8 and *RUNX1* mutation, and absence of complex karyotype, *FLT3/ITD* or *NPM1* mutations;[76] the presence of *ASXL1* mutations did not carry an independent prognostic value in terms of survival. In another study involving patients with CMML, the mutation was reportedly associated with poor prognosis.[77]

The results of the above studies are clouded by the possibility that the most frequent mutation (c.1934dupG; p.Gly646TrpfsX12) in virtually all the studies might be an artifact of PCR amplification.[78] In a recent study that took this possibility into account, *ASXL1* mutational frequencies were 13% in PMF, 23% in post-PV/ET MF, 18% in blast-phase MPN and 20% in CMML.[39] The same study demonstrated co-occurrence of mutant *ASXL1* with *TET2*, *JAK2*, *EZH2*, *IDH* and *MPL* mutations. *ASXL1*-mutated PMF patients were cytogenetically normal and none underwent leukemic transformation during follow-up; the presence of mutant *ASXL1* in PMF did not have an independent prognostic effect.[39] Similarly, the three *ASXL1*-mutated CMML cases were alive after 40, 34 and 12 months from the time of mutation analysis and none of them had progressed to acute leukemia.[39] Other *ASXL1*-related abstracts that were presented at ASH 2010 included c.1934dupG;p.Gly646TrpfsX12 as a true mutation and reported much higher mutation prevalence in PMF and post-MDS/CMML AML.[79,80]

IDH mutations

IDH1 and *IDH2* map to chromosomes 2q33.3 and 15q26.1, respectively. *IDH* mutations involve exon 4, are heterozygous and affect three specific arginine residues: R132 (*IDH1*), R172 (the *IDH1* R132 analogous residue on *IDH2*) and R140 (*IDH2*).[35] *IDH* mutations induce loss-of-activity for the conversion of isocitrate to 2-ketoglutarate and gain-of-function in the conversion of 2-ketoglutarate to 2-hydroxyglutarate.[81,82] Consistent with these observations, heterozygous germ-line mutations in *IDH2*R140 occur in patients with neurometabolic disease and 2-hydroxyglutarate aciduria.[83] The 2-hydroxyglutarate might be the mediator of impaired TET2 function in cells with mutant *IDH* expression.[68]

IDH1 and *IDH2* mutations were first described in gliomas.[84] Several studies have since reported on the occurrence of *IDH* mutations in both primary and secondary AML. In one of the most recent studies involving 446 adult Chinese patients with non-M3 primary AML,[85] ~9% harbored *IDH2*R140, ~6% *IDH1*R132 and ~3% *IDH2*R172 mutations. Mutant *IDH2* clustered with intermediate-risk or normal karyotype and isolated trisomy 8, but not with *WT1* mutations or core-binding factor AML;[85] the presence of *IDH2* mutations was prognostically favorable and *IDH2*R172 was mutually exclusive of *NPM1* mutations.[85] The association of *IDH* mutations with trisomy 8 was formally examined in 157 patients with myeloid malignancies associated with isolated trisomy 8;[86] 18 *IDH* mutations were identified, including 15 *IDH2* (14 R140Q) and 3 *IDH1* mutations. *IDH1*/*IDH2* mutational frequencies in the particular study were 27% for post-MDS AML, 25% for therapy-related MDS/AML, 15% for *de novo* MDS, 13% for *de novo* AML and 3% for MPN. By comparison, *IDH* mutational frequencies were significantly lower among patients with AML or MDS without isolated trisomy 8.[86]

At ASH 2010, an Eastern Cooperative Oncology Group (ECOG) Study of 398 young (<60 years old) patients with *de novo* AML reported 8% *IDH2* and 6% *IDH1* mutations;[87] 10% had *TET2* and 4% *ASXL1* mutations. In this ECOG Study, mutual exclusivity was demonstrated for *IDH* and either *TET2* or *WT1* mutations and *FLT3* and *ASXL1* mutations;[87] survival was favorably affected by the presence of *IDH2*R140Q or *CEBPA* and absence of *FLT3* or *ASXL1* mutations. Another study had suggested an association between *IDH1* and *NPM1* mutations and a negative prognostic effect from *IDH1* mutations for relapse in *FLT3/ITD*$^-$patients and a favorable effect in *FLT3/ITD*$^+$ cases.[88] In yet another study, *IDH* mutations were significantly associated with normal karyotype and *IDH1* mutations clustered with *NPM1* but not *CEBPA* mutations and predicted inferior prognosis, in the absence of *FLT3/ITD* mutations; *IDH2*-mutated patients with normal karyotype also had poor prognosis.[89] Other studies have also found the adverse prognostic impact of *IDH* mutations in *NPM1*$^+$ *FLT3/ITD*$^-$ AML with normal karyotype.[90]

The largest study of *IDH* mutation analysis in MPN involved 1473 patients and reported *IDH* mutational frequencies of ~2% in PV, 1% in ET, 4% in PMF and 22% in blast-phase MPN.[35] In this study, a total of 38 *IDH* mutations were detected: 18 *IDH1*R132, 19 *IDH2*R140 and 1 *IDH2*R172. Mutant *IDH* was

documented in the presence or absence of *JAK2*, *MPL* and *TET2* mutations. *IDH*-mutated patients were more likely to be nullizygous for *JAK2* 46/1 haplotype and less likely to display complex karyotype.[35] In blast-phase MPN, but not chronic-phase PMF, *IDH* mutational status predicted poor survival. The relatively high incidence of *IDH* mutations in post-MPN/MDS AML has also been noted in other studies.[37–39] In most of these studies, paired sample analysis did not suggest acquisition of *IDH* mutations during leukemic transformation. The frequency of *IDH* mutations was also relatively high (~22%) in high-risk as opposed to low-risk MDS/AML (0%) associated with isolated del(5q).[91,92] In another study of 100 MDS, 90 MDS/MPN (including 88 CMML) and 41 post-MDS/MPN cases, *IDH1* ($n=4$) or *IDH2* ($n=13$) mutational frequencies were 5% in MDS, 9% in MDS/MPN and 10% in post-MDS/MPN AML.[55]

EZH2 mutations

EZH2 maps to chromosome 7q36.1. Wild-type EZH2 is part of a histone methyltransferase (polycomb repressive complex 2 associated with H3 Lys-27 trimethylation) and is overexpressed in solid tumors.[93] Morin *et al*. were the first to report on somatic *EZH2* mutations involving exon 15 (*EZH2*Y641F/N/H/S), with mutational frequencies of ~22% in germinal center B-cell diffuse large B-cell lymphomas and 7% in follicular lymphomas.[94] It was subsequently shown that *EZH2*Y641F/N represents a dominant gain-of-function mutation and promotes H3 Lys-27 trimethylation.[95,96]

Initial reports of *EZH2* mutations in myeloid malignancies involved patients with MDS,[97] MPN or MDS/MPN. The MDS Study[97] involved 126 patients and showed *EZH2* missense, donor splice-site or frameshift mutations, involving exons 7, 8, 10, 17 and 18 and intron 19 in 8 (6%) patients. Three patients had biallelic mutations. In addition, the *EZH2* locus at 7q36.1 was deleted at one allele in 22 patients, raising the frequency of point mutations or deletions to 23%, of which 40% also displayed *TET2* mutations.[97] In another primarily non-MPN Study,[98] a total of 344 patients were studied: 131 MDS, 89 primary AML, 83 MDS/MPN, including 25 CMML, 24 secondary AML and 17 MPN. Exon 18/19 mutations were detected in three MDS/MPN, including two (8%) CMML, two (1.5%) MDS and one (1%) primary AML cases.[98] Mutational frequencies were 20% in patients with 7q UPD (uniparental disomy) and 7% in those with del(7q).[98]

Ernst *et al*.[41] were the first to report on the occurrence of *EZH2* mutations in MPN and MDS/MPN[41] They studied a total of 624 patients: 154 MDS including 2 post-MDS AML, 219 MDS/MPN including 118 with CMML, 90 with classic MPN including 30 each with PV, ET or MF, 67 with other MPN including 30 each with systemic mastocytosis (SM) or hypereosinophilic syndrome/chronic eosinophilic leukemia, 54 AML with −7/del(7q) and 40 blast-phase CML. They found 49 mutations in 42 patients, including 9 among 12 patients with 7q UPD. Mutational frequencies were 13% in CMML, 13% in atypical CML, 13% in MF (PMF or post-PV/ET MF), 10% in MDS/MPN-U, 6% in MDS, 3% in PV and 3% in hypereosinophilic syndrome/chronic eosinophilic leukemia.[41] Also in this study, co-occurrence of *EZH2* and *TET2* mutations was documented with mutant *EZH2* being the first to appear. All patients with −7 or 7q UPD were homozygous or hemizygous for *EZH2* mutations, whereas 9 of 12 7q UPD-negative patients were heterozygous. *EZH2* variants in this study included missense, frameshift or stop mutations expected to result in premature chain termination or truncation of critical domains;[41] protein blotting revealed absent trimethylated H3 Lys-27 (H3K27me3) in cell lines with mutant *EZH2* and decreased EZH2 catalytic activity in insect cells infected with mutant *EZH2*.[41] Taken together, the observations from the study by Ernst *et al*.[41] suggest a tumor suppressor activity for MPN-associated *EZH2* mutations, which contrasts with the gain-of-function activity for the lymphoma-associated *EZH2*Y641F/N/H/S.[93]

At ASH 2010, several studies of *EZH2* mutations in myeloid malignancies were presented by other investigators. Abdel-Wahab *et al*.[39] studied 94 patients, including 46 with PMF, 22 post-PV/ET MF, 11 blast-phase MPN and 15 CMML, for EZH2, ASXL1, *TET2*, *IDH*, *JAK2* and *MPL* mutations. *EZH2* mutations were seen in three (7%) patients with PMF and coexisted with mutant *ASXL1* in one patient. All *EZH2*-mutated PMF patients had normal karyotype and none underwent leukemic transformation during follow-up. Steglemann *et al*.[99] studied 62 patients with PMF, 21 with post-ET/PV MF and 6 post-MPN AML with chromosome 7q abnormality. They found 10 *EZH2* mutations in eight patients: six (~10%) PMF and one each with post/PV/ET MPN and post-MPN AML. Two of their PMF cases displayed double *EZH2* mutations, and co-occurrence of *EZH2* and *JAK2* mutations was also documented. It is premature at the present time to comment on clinical correlates or the prognostic effect of *EZH2* mutations in myeloid malignancies.

UTX, located on chromosome Xp11.2, is a H3K27me3 demethylase, also belonging to the polycomb group of proteins.[100] *UTX* mutations were first described by van Haaften *et al*.[101] in multiple cancer types, including multiple myeloma, gastrointestinal cancers and myeloid leukemias. These mutations were described as being inactivating, homozygous, heterozygous or hemizygous, and constituting frameshift, missense or stop codon mutations. *UTX* mutations have recently been reported also in MDS/MPN, including CMML, MDS (refractory anemia with excess blasts-1) and secondary AML.[26,102,103] There occurrence in MPN and precise pathogenetic contribution in general remain to be further elucidated.

Prognostic studies

ET

The WHO classification system underscores the difference between ET and prefibrotic PMF.[1] The two are distinguished based on bone marrow morphology; in ET, megakaryocytes are large, hyperlobulated and mature –appearing, whereas in prefibrotic PMF, they are immature appearing with hyperchromatic and irregularly folded bulky nuclei.[104,105] Furthermore, megakaryocyte changes in prefibrotic PMF are accompanied by left-shifted granulocyte proliferation, which is usually not the case in ET.[106]

Barbui *et al*.[107] looked into the prognostic relevance of the distinction between ET and prefibrotic PMF in an international study of 1104 patients previously diagnosed and treated as ET. Central review of the bone marrow biopsies according to the WHO morphological criteria confirmed ET in 81% of the patients, and diagnosis was revised to early, prefibrotic PMF in 16%. Early PMF, as opposed to ET, was characterized by significantly higher leukocyte and platelet counts, lower hemoglobin level, higher serum lactate dehydrogenase level, higher circulating CD34+ cell count and a higher incidence of palpable splenomegaly. Patients with early PMF, as compared with those with ET, were more likely to develop overt myelofibrosis and acute leukemia. Cumulative leukemic transformation rate at 10 and 15 years was 0.7 and 2.1% in ET versus 5.8 and 11.7% in early/prefibrotic PMF, respectively. The

10- and 15-year overall survival rates were 89 and 80% in ET versus 76 and 59% in early/prefibrotic PMF, respectively. This study validates the clinical relevance of strict adherence to WHO criteria in the diagnosis of ET.[108] The study also confirms the clinically indolent nature of ET with near-normal life expectancy and a less than 1% risk of leukemic or fibrotic transformation in the first 10 years of disease.

PMF

The International Prognostic Scoring System (IPSS) for PMF uses five predictors of inferior survival: age >65 years, hemoglobin <10 g/dl, leukocytes $>25\times10^9$/l, circulating blasts ⩾1% and constitutional symptoms.[109] The Dynamic IPSS (DIPSS) utilizes the same prognostic variables but can be applied at any time during the disease course.[110] At the 2010 ASH meeting, Gangat *et al*.[111] presented a new prognostic model for PMF that is now published in full. The new model is called DIPSS-plus and incorporates DIPSS-independent prognostic factors, including unfavorable karyotype,[112] red cell transfusion need[113,114] and platelets $<100\times10^9$/l.[115] In another paper presented at the ASH 2010, Caramazza *et al*.[112] described the following cytogenetic abnormalities as being unfavorable to both overall and leukemia-free survival in PMF: complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p- or 11q23 rearrangement.

The DIPSS-plus prognostic model was developed using 793 PMF patients seen at the Mayo Clinic and uses eight instead of five risk factors to define low- (no risk factors), intermediate-1- (one risk factor), intermediate-2- (two or three risk factors) and high (four or more risk factors)-risk disease;[111] the corresponding median survivals were 185, 78, 35 and 16 months ($p<0.0001$). Multivariable analysis identified platelet count and karyotype as independent predictors of leukemia-free survival. Other risk factors that are worthy of further investigation in PMF include nullizygosity for JAK2 46/1 haplotype,[18,35] low JAK2V617F allele burden[116,117] and increased plasma levels of interleukin (IL)-8, IL-10, IL-15 or IL-2R.[112] The latter work was also presented at ASH 2010.[118] The study used a multiplex biometric sandwich immunoassay to measure plasma levels of 30 cytokines in 127 patients with PMF and showed DIPSS-independent inferior survival in patients, with increased levels of IL-2R, IL-8, IL-15 and CXCL10.

Clinical trials in MPNs

JAK inhibitor treatment trials

The two noteworthy JAK inhibitor clinical trials presented were those of Pardanani *et al*.[119] where CYT387, a JAK1/2 inhibitor, was used in MF and Verstovsek *et al*.[120] where INCB018424 (JAK1/2 inhibitor) was used in hydroxyurea-refractory or intolerant PV or ET. In the former study, 36 MF patients received CYT387 in a phase-1/2 study and were followed for a median of 15 weeks. Dose-limiting toxicity was established at 400 mg/day and included asymptomatic grade 3 hyperlipasemia or grade 3 headache. Maximum tolerated dose for CYT387 was declared at 300 mg/day. Grade 3/4 non-hematologic adverse events were infrequent and included asymptomatic elevations of liver function tests and pancreatic enzymes. A unique side effect of the drug, characterized by lightheadedness and hypotension, occurring only with the first dose was documented in 36% of patients. Grade 3/4 thrombocytopenia was seen in 22% of patients and grade 3 anemia in 3%.

Anemia response to CYT387, according to the International Working Group for MPN Research and Treatment (IWG-MRT) criteria, was documented in 41% of MF patients. The drug induced transfusion independency in an even higher percentage of patients. Almost all (97%) patients experienced reduction in spleen size, which was >50% in 11 (37%) patients. The drug was also effective in controlling constitutional symptoms, including pruritus, in the majority of patients. Of note, treatment responses were also seen in patients who previously failed treatment with pomalidomide[121] or other JAK inhibitors, such as INCB018424[122] or TG101348.[123] Anemia response was not affected by the presence of *JAK2*V617F. It is important to note that CYT387 is the first JAK inhibitor demonstrating substantial activity against MF-associated anemia and that anemia is the chief determinant of quality of life in MF and is also the most important prognostic factor for survival.[109–111,113]

Verstovsek *et al*. presented longer-term follow-up of an ongoing trial of INCB018424 in hydroxyurea-refractory or intolerant PV or ET. Starting doses were 10 and 25 mg b.i.d. The study included 34 PV patients followed up for a median of 15 months. Almost all (97%) patients became phlebotomy independent. A greater than 50% reduction in spleen size was achieved in 59% of patients. Leukocytosis or thrombocytosis resolved in 63 and 69% of patients, respectively. Six (18%) patients discontinued therapy. Seven grade 3 adverse events were reported and included thrombocytopenia and neutropenia. Similarly, a total of 39 ET patients were enrolled and followed for a median of 15 months. Normalization of platelet count occurred in 49% of these patients after a median of 2 weeks. Nine (23%) patients discontinued therapy. Grade 3 adverse events included leukopenia in two patients and peripheral neuropathy in one. As expected, the drug controlled pruritus and other constitutional symptoms in the majority of patients. Only 6% of PV and 12% of ET patients experienced a >50% decrease in *JAK2*V617F allele burden.

PV or ET patients who are either intolerant or resistant to hydroxyurea are effectively managed by interferon-α,[124,125] busulfan[126,127] or pipobroman (not available in the United States).[128–131] Among these second-line drugs, interferon-α is usually used for patients younger than 65 years of age and busulfan in the older age group. All three second-line drugs induce phlebotomy independence in almost all patients with PV, and response rates for thrombocytosis or leukocytosis often exceed 80%, which is superior to what was mentioned above for INCB018424. The suggestion that busulfan or pipobroman might be leukemogenic in PV or ET is completely unfounded and is often used as a scare tactic to promote the use of new drugs.[124–126,132–135]

Other treatment trials in myelofibrosis

Begna *et al*.[136] presented results from a phase-2 study using single-agent low-dose (0.5 mg/day) pomalidomide in anemic patients with MF. The main eligibility criterion was transfusion dependence or hemoglobin <10 g/dl; subjects failing previous treatment with lenalidomide or thalidomide were eligible. A total of 58 patients were included in the study, among whom 46 (79%) were transfusion dependent and 42 (72%) were *JAK2*V617F positive. Treatment was well tolerated, with no instances of thrombosis. There was grade1 neuropathy possibly related to drug in one subject. Grade 3 thrombocytopenia/neutropenia occurred in two subjects. Anemia response, per IWG-MRT criteria, was seen in 10 (17%) subjects including 9 who became transfusion independent. In addition, 14 of 24 patients (58%) with platelets $<100\times10^9$/l had a >50%

increment in their platelet count. There were no spleen responses. Anemia response occurred only in the presence of *JAK2*V617F (24 versus 0%; $P=0.03$), and was predicted by the presence of pomalidomide-induced basophilia in the first month of therapy. Accordingly, pomalidomide should be a valuable treatment option for anemia in *JAK2*V617F-positive patients with MF in the absence of marked splenomegaly.[121,136]

Vannucchi *et al*.[137] presented results from a phase-1/2 study of RAD001, an oral inhibitor of mammalian target of rapamycine in PMF and post-PV/ET MF. A total of 30 patients were treated with 10 mg daily, which was considered as the maximum tolerated dose. Non-hematologic toxicity included frequent grade 2 mouth ulcers and grade 1/2 hypertriglyceridemia. Grade 3/4 hematological toxicities included anemia in four patients and thrombocytopenia in one patient. According to IWG-MRT criteria, six (23%) patients experienced clinical improvement, which includes >50% spleen size reduction or anemia response. In addition, 11 (52%) of 21 patients had complete resolution of systemic symptoms and 14 (74%) of 19 patients reported disappearance of pruritus. The drug did not affect *JAK2*V617F allele burden. A set of 46 inflammatory protein markers and cytokines were quantified and some, including IL-10 and MIP-1b, showed significant decrease, whereas others, including factor VII, IL-8 and matrix metalloproteinase-2, showed an increase in post-treatment samples. *JAK2*V617F activates STAT3/5, RAS/MAPK and PI3/AKT pathways. It is therefore rationale to target the PI3/AKT and mammalian target of rapamycine pathways, and *in vitro* studies have demonstrated the therapeutic potential of such a strategy.[138,139] The study by Vannucchi *et al*.,[137] serves as proof-of-concept in this regard.

Treatment for mastocytosis

Gotlib *et al*.[140] presented results of a phase-2 study in SM using midostaurin (PKC412), an inhibitor of wild-type and D816V KIT. PKC412 was orally administered to 26 patients at 100 mg b.i.d.[140] Major response rate per conventional criteria was 38% and benefits included normalization of hypoalbuminemia, improvement of hemoglobin and platelet counts, resolution of liver function abnormalities, improvement of pleural effusion and ascites, and reversion of weight loss. Some of these responses were accompanied by improvement in hepatosplenomegaly, a >50% decrease in serum tryptase level and/or marrow mast cell burden, and improvement in mediator symptoms. One patient with mast cell leukemia had achieved a near-complete remission, with a decrease of serum tryptase from 763 to <20 ng/ml and decrease of marrow mast cell burden from 60–70% to 5%. The most common drug side effects were nausea, vomiting, diarrhea and fatigue. Asymptomatic hyperlipasemia occurred in five patients.

Hermine *et al*.[141] presented results on 44 patients with mastocytosis treated with cladribine. Cladribine was given at 0.15 mg/kg/day in a 2-h infusion or subcutaneously for 5 days, repeated every 1–2 months, for a median of four cycles. After a median follow-up of 35 months, no opportunistic infections were seen, with the exception of zoster infections in two patients. Responses occurred in 24/31 patients with urticaria pigmentosa, 17/35 with fatigue, 14/24 with flushing, 9/24 with pruritus, 9/21 with abdominal pain, 1/9 with ascites, 11/23 with diarrhea, 8/16 with weight loss, 4/14 with headache, 5/10 with cough, 7/20 with splenomegaly, 2/6 with lymphadenopathy, 0/2 with pleural effusions and 5/19 with neuropsychological symptoms. In addition, eosinophil count normalized in 7/10 cases and a substantial decrease in tryptase levels was also noted. Overall, major and partial responses were observed in 7/12 patients with aggressive SM, 3/3 smoldering SM, 17/19 indolent SM, 2/3 cutaneous mastocytosis but in none of the patients with SM associated with another myeloid malignancy.

The above study by Hermine *et al*.[141] validates the value of cladribine in SM[142] and provides clinically useful information on where the drug works best in terms of SM variant and specific symptom. However, the results look different than those recently published from the Mayo Clinic.[143] The Hermine study suggests that cladribine might not be effective in SM associated with another myeloid malignancy, whereas the response rate in this SM variant was reported at 46% in the Mayo Clinic study. Similarly, the response rates for the other SM variants were substantially higher than those reported by the Mayo investigators. Regardless, in the Mayo Clinic study,[143] presence of leukocytosis, monocytosis or circulating immature myeloid cells was significantly associated with inferior response to cladribine.

As was well demonstrated by Gotlib *et al*.,[140] midostaurin therapy has the potential to produce substantial reduction in mast cell burden in some patients with SM. However, it is currently not clear which patients with SM stand to benefit from such treatment, and more studies are needed to clarify the advantage of midostaurin over treatment with cladribine.[143] Of note, cladribine has also been successfully used in mast cell leukemia.[144] Interferon (IFN)-α is another useful drug for the treatment of SM. In a recent Mayo Clinic study, IFN-α induced a response rate that was 41% and more likely to occur in the absence of anemia or elevated erythrocyte sedimentation rate.[143] Taken together, midostaurin therapy might be most useful in the treatment of aggressive SM or mast cell leukemia, especially if combined with either cladribine or IFN-α.

IFN-α therapy in PV or ET

Quintas-Cardama *et al*.[145] presented a phase-2 study of subcutaneous pegasys (peginterferon-α-2a) in 84 patients with PV or ET. Initial dose was 450 mcg/week, which was subsequently modified to 90 mcg/week. After a median follow-up of 40 months, complete remission rate was 75%. Of five patients with abnormal karyotype at study entry, two reverted to diploid cytogenetics. Overall, 28% of patients had a >50% reduction in *JAK2*V617F allele burden and 19% had complete molecular response. *TET2* or *ASXL1* mutational status did not appear to impact the likelihood of achievement of molecular response. In all, 25 (30%) patients were taken off study after a median of 9 months and the reason in half of them was drug toxicity, including anorexia, depression, fatigue, ischemic retinopathy, dyspnea and neuropathy. The results of this study support the use of pegasys in hydroxyurea-refractory PV or ET. However, controlled studies are needed to assess the value of the drug as first-line therapy. IFN-α can induce molecular remissions in 10–20% of patients with PV, but what exactly this means in terms of overall outcome is not clear.

Conclusions

There is no doubt that more mutations in MPN will be described in the coming years. However, it is difficult to say at this point that we are that much more enlightened about disease pathogenesis. Similarly, the concept of targeted therapy in MPN is proving to be more complicated than expected, and whether or not the recent description of several epigenetically-implicated mutations supports continued evaluation of DNA methyltransferase or histone deacetylase inhibitors is not clear. Nevertheless, one cannot deny the benefit of new drugs such as pomalidomide and JAK inhibitors, even though we are uncertain

about their precise mechanism of action. In the near future, we foresee the incorporation of molecular or biological markers in disease prognostication and monitoring of treatment response, whereas ongoing phase-3 studies will better define the therapeutic role of JAK inhibitors, pomalidomide and IFN-α.

Conflict of interest

The authors declare no conflict of interest.

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