Leukaemic Transformation of Philadelphia-chromosome-negative Myeloproliferative Neoplasms – A Review of the Molecular Background

Nils H Thoennissen and H Phillip Koeffler

Abstract

Philadelphia-chromosome-negative myeloproliferative neoplasms (MPNs), including polycythaemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythaemia (ET), are clonal haematopoietic stem cell disorders characterised by proliferation of one or more myeloid cell lineages. They are closely associated with the JAK2V617F mutation, whose detection is used as a clonal marker in the differential diagnosis of MPN. Despite recent improvements in the molecular diagnosis and therapeutic regimen of these chronic disorders, haematological evolution to blast phase remains a major cause of long-term mortality. The mechanism of MPN transformation is still a matter of some controversy because of insufficient insights into the underlying molecular pathogenesis. The purpose of this article is to summarise the increasing data concerning the mechanism of leukaemic evolution of patients diagnosed with chronic MPN. Chromosomal abnormalities and genes that have been shown to play a potential causative role in chronic-phase acceleration are discussed, as are aberrations that may serve as prognostic markers in the blast phase of MPN.

Keywords
Philadelphia-chromosome-negative myeloproliferative neoplasms, leukaemic transformation

Philadelphia-chromosome-negative myeloproliferative neoplasms (MPNs), including polycythaemia vera (PV), primary myelofibrosis (PMF) and essential thrombocythaemia (ET) are defined as clonal haematopoietic stem cell disorders. They are characterised by increased proliferation of terminally differentiated myeloid cells.

In recent years, much progress has been made in understanding the molecular origin of these chronic entities. The tyrosine kinase JAK2 and thrombopoietin receptor (MPL) were directly linked to the pathogenesis of MPN with the identification of somatic mutations including JAK2V617F, or rare variants, as well as MPLS515. These different mutant alleles all result in a gain of function due to the constitutive activation of tyrosine kinase-dependent cellular signalling pathways. Recently discovered deletions or mutations involving TET2, ASXL1 and IDH1/2 shed more light on possible mechanisms of excessive proliferation in chronic MPN. Among the classic MPNs, PV and ET are relatively indolent disorders, resulting in a modest reduction of lifespan compared with a control population; however, most patients ultimately suffer from one or more severe and potentially fatal complications directly attributable to the disease. By comparison, in most cases PMF has a more severe course and survival is significantly affected.

Nonetheless, MPN patients are at a significantly increased risk of transformation to acute myelogenous leukaemia (AML), which is generally associated with a dismal prognosis. The incidence of chronic-phase MPN is two to five cases per 100,000 population per year, whereas leukaemic evolution occurs in 2–5% of these patients with either PV or ET and roughly 20% of cases with PMF. Patients with de novo and secondary AML have a similar spectrum of structural abnormalities, including specific chromosomal sites. The occurrence of cytogenetic changes associated with unfavourable risk such as -7/7q- or complex karyotype is higher in secondary AML. These changes are most probably induced by the type of therapy given during the chronic phase of MPN. Apart from the chromosomal alterations, cumulative insights have been made into the causative genes that underlie the leukaemic transformation of chronic MPNs.

Recent findings have suggested that transition from heterozygosity to homozygosity for JAK2V617F is associated with an increased hyperproliferative disease and may be important for disease progression, at least from PV to secondary myelofibrosis. Moreover, one longitudinal prospective study suggested that the presence of a JAK2V617F haematopoietic clone was significantly associated with leukaemic transformation in PMF. The role of JAK2V617F in clonal evolution is of particular interest as the examination of its kinetics could potentially allow the prediction of disease acceleration. It could also determine whether treatment with novel JAK2 inhibitors during the chronic phase of MPN may be effective in reducing the incidence of transformation. However, our group at the Cedars-Sinai Medical Center showed no statistical difference between the time to transformation in MPD patients with homozygous JAK2V617F
compared with patients with heterozygous JAK2V617F or wild-type JAK2 genes.8 No disease progression was observed between the JAK2V617F(-) chronic phase and JAK2V617F(+) blast phase of MPN. Additionally, several other groups have reported a decrease in allelic burden or even a complete disappearance of JAK2V617F at the time of leukemic transformation.18,21 This is in accordance with Cedars-Sinai Medical Center findings showing that 18% of chronic MPN samples that were initially positive for either heterozygous or homozygous JAK2V617F became negative for these abnormalities in the blast phase.23 Taken together, the initial aberrant clone of MPN cells may be JAK2V617F(-) with a higher propensity to undergo clonal evolution, whereas this evolution particularly favours the clone acquiring the JAK2V617F during the chronic phase of MPN. Therefore, the presence of this mutation does not appear to be a prerequisite for the leukemic transition of MPN, suggesting that additional genetic events are required for full transformation.

One of the molecular mechanisms associated with leukemic evolution was identified almost two decades ago in a relatively small cohort of MPN patients.22,23 Aberrations of the tumour suppressor gene TP53 were associated with prior exposure of these patients to genotoxic agents and leukemic evolution to MPN blast phase. These individuals had mainly been treated with alkylating agents, including chlorambucil and busulfan, as well as radio-therapeutic regimens (e.g. 32P). Both therapies are known to cause DNA damage together with a significant rate of TP53 aberrations.21 It was also noted in patients who underwent leukemic evolution that either loss of heterozygosity (LOH) at 17p or mutation of TP53 was related to prior exposure to hydroxyurea, a non-alkylating agent.26,27 However, large prospective studies of MPN patients concluded that the leukemogenic potential of this cytoxic drug compound by itself is low, whereas the risk increases when hydroxyurea is combined with other chemotherapy drugs known to damage DNA.2,17,24 Nevertheless, individuals with MPN who progress to AML may have an aberrant TP53 gene in the absence of exposure to a known mutagen. This is consistent with TP53 alterations being associated with the clonal evolution of many other types of cancers.29

Missense mutations involving the dominantly acting RAS gene family and their signal transduction pathway occur frequently in MPN.8 However, individuals with MPN who progress to AML may have an aberrant TP53 gene in the absence of exposure to a known mutagen. This is consistent with TP53 alterations being associated with the clonal evolution of many other types of cancers.29

Our Cedars-Sinai Medical Center team performed a high-density single nucleotide polymorphism (SNP)-array analysis in a large cohort of 148 patients in the chronic or blast phase of MPN to obtain a profile of genomic alterations and gain more insight into the molecular processes of leukemic transformation.27 Confirming the hypothesis that evolution from MPN to leukemia is accompanied by the acquisition of additional genomic lesions, two to three times more aberrations were detected in the blast phase compared with the chronic phase of MPN, independently of JAK2V617F. Notably, ET patients had fewer alterations in their chronic-phase samples compared with the PV and PMF cases, whereas the number was comparable in all three MPN subgroups after their transformation. Altered regions commonly occurred on chromosomes 12p (ETV6) and 21q (RUNX1) as well as on 17p (TP53). These genes are known to be involved in the development of de novo as well as secondary AML following myelodysplastic syndrome.28-31 It was shown in two case studies that a relatively high number of MPN patients (28 and 37.5%, respectively) acquired a mutation in the RUNX1 gene at the time of disease transformation.28-31 Notably, transduction of an expression vector carrying mutant RUNX1 into CD34+ cells from MPN patients markedly promoted their proliferation, independently of the presence of JAK2V617F.24 Moreover, our group found gain of chromosomal material at 8q24.21 (c-MYC), including trisomy 8, almost exclusively in the JAK2V617F(-) transformed samples.25 This suggests that the increased oncogenic activity of c-MYC is associated with a strong selection of leukemic clones that can almost completely displace the myeloproliferative compartment with the JAK2 gain-of-function mutation.28

In addition to aberrations involving known oncogenes (c-MYC) or tumour suppressor genes (ETV6, TP53, RUNX1) with already-established leukemogenic potential, frequently altered regions were detected by SNP-Chip on chromosomes 1q, 7q, 16q, 19p and 21q in MPN blast-phase samples, which may harbour promising new candidate genes.32 Abnormalities involving chromosome 7 are frequently detectable in de novo and secondary AML. Preceding studies have found a critical breakpoint region involving a locus at centromeric band 7q22, whereas the telomeric breakpoint varies from q32 to q36.33-36 Interestingly, the minimal deleted region in our SNP-array study was located at 7q22.1, encompassing only two target genes: SH2B2 and CUTL1. SH2B2 regulates and enhances JAK2-mediated cellular responses,37 and the CUTL1 gene encodes for a CUT family member of the homeodomain proteins that can repress the expression of developmentally regulated myeloid genes.38 Moreover, genome-wide inspection for minimal regions of duplications/amplifications revealed several interesting genes including PIN1, ICAM1 and CD137 on 19p as well as ERG on 21q. Whereas the latter three targets have been shown to possess potential pro-growth activity in de novo AML and/or myelodysplastic syndrome.39-41 PIN1 is known to be overexpressed in a variety of cancers. It may act as an oncogene via promotion of cell-cycle progression and proliferation.42 However, whether such lesions impart disease-promoting activity or reflect genetic instability during clonal progression in MPN is currently not delineated.

The identification of novel genetic lesions in MPN will probably not only provide new specific diagnostic, prognostic and therapeutic tools, but also increase understanding of the pathogenesis of the disease and its potential acceleration to AML. Recently, TET2, a member of the ten-eleven-translocation (TET) family of genes, has been found to be mutated in various haematopoietic disorders.43-45 TET2 mutational frequencies did not differ across the MPN subcategories (13–17%) and appeared independently of JAK2V617F. Comparable to family member TET1, TET2 inactivation may have epigenetic consequences that deregulate genes involved in early haematopoiesis as well as in myeloid differentiation.46 While mutant TET2 may co-operate with other mutated genes to induce AML, the potential value of the mutation as a prognostic tool appears to be low, since no correlation has been found between the presence of mutant TET2 and survival or leukemic transformation.47 The ASXL1 gene is mutated in approximately 20% of chronic- or blast-phase MPN cases. Analysis of a cell line from patients with MPN that evolved into AML demonstrated that these mutations were always present during the chronic phase of the
pathogenesis of MPN and other myeloid malignancies, but did not appear to be acquired during leukemic transformation.\(^4\)

Gene family members IDH1 and IDH2, which encode enzymes that catalyse oxidative decarboxylation of isocitrate to α-ketoglutarate, have also been found to be mutated in patients with myeloid disorders.\(^5\) Since intact isocitrate dehydrogenase IDH activity is required for cellular protection from oxidative stress by the generation of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione, it is believed that both IDH1 and IDH2 act as tumour suppressors. Recently, a multi-institutional project performed a study on a large number of MPN patients in different stages of the disease, and a relatively infrequent number of IDH1 and IDH2 mutations were found in samples from the chronic phase.\(^6\) By contrast, the IDH mutational rate was significantly more prevalent in the blast phase of MPN.\(^7\) \(^8\) The IDH1 and IDH2 mutations, which encode enzymes that catalyse oxidative decarboxylation of isocitrate to α-ketoglutarate, have also been found to be mutated in patients with myeloid malignancies.\(^9\) Since intact isocitrate dehydrogenase IDH activity is required for cellular protection from oxidative stress by the generation of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione, it is believed that both IDH1 and IDH2 act as tumour suppressors. Recently, a multi-institutional project performed a study on a large number of MPN patients in different stages of the disease, and a relatively infrequent number of IDH1 and IDH2 mutations were found in samples from the chronic phase.\(^6\) By contrast, the IDH mutational rate was significantly more prevalent in the blast phase of MPN.\(^7\) \(^8\) The IDH1 and IDH2 mutations, which encode enzymes that catalyse oxidative decarboxylation of isocitrate to α-ketoglutarate, have also been found to be mutated in patients with myeloid malignancies.\(^9\) Since intact isocitrate dehydrogenase IDH activity is required for cellular protection from oxidative stress by the generation of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione, it is believed that both IDH1 and IDH2 act as tumour suppressors. Recently, a multi-institutional project performed a study on a large number of MPN patients in different stages of the disease, and a relatively infrequent number of IDH1 and IDH2 mutations were found in samples from the chronic phase.\(^6\) By contrast, the IDH mutational rate was significantly more prevalent in the blast phase of MPN.\(^7\) \(^8\)

Concerning the prognostic impact of genetic lesions, LOH caused by deletion of chromosome 17p (TP53) is significantly associated with a complex karyotype and poor survival in myeloid malignancies, including blast transformation of MPN.\(^10\) Recently, copy-number neutral (CNN)-LOH has been frequently detected in myeloid malignancies. It is defined as the presence of a chromosomal pair that derives from only one parent in a diploid individual. It cannot be detected by cytogenetics. Acquired CNN-LOH occurs as a result of a mitotic error in somatic cells. This is an important step in cancer development and progression due to the production of homozygosity (caused by mutated or methylated genes) or an aberrant pattern of imprinting. It is well-known in chronic-phase Philadelphia-positive MPN that the activating mutation JAK2V617F is strongly associated with the common 9p CNN-LOH, which uncovered a new paradigm that a dominant oncogenic mutation may be further potentiated by duplication of the mutant allele and/or exclusion of the wild-type allele.\(^11\) CNN-LOH can involve chromosomal regions that are also frequently affected by deletions. This may have prognostic implications at least similar to the deletions visible by karyotyping. Using SNP-array analysis as a robust and detailed approach to detect CNN-LOH, we found 9p CNN-LOH with homozygous JAK2 mutation associated with an inferior outcome in MPN blast crisis in comparison with individuals with either heterozygous JAK2V617F or wild-type JAK2.\(^12\) In contrast to LOH on 17p, the prognostic impact of 9p CNN-LOH was independent of established risk factors such as -7/-7q, complex karyotype and -5/-5q. Although JAK2V617F in association with 9p CNN-LOH appears to have no direct impact on MPN transformation, we suggest that the homozygous driver mutation in combination with additional newly acquired aberrations in terms of an additional hit may influence the clinical course of patients with MPN blast phase. As expected in our SNP-array study, blast-phase patients with loss of chromosomal material on 9q showed a significantly worse outcome than patients with homozygous driver mutation in combination with additional newly acquired aberrations in terms of an additional hit. Since this is known to be predictive for rapid progression and reduced response in AML therapy,\(^13\) \(^14\) interestingly, MPN blast-phase patients with 9q CNN-LOH had comparable survival rates to those with -7/-7q in their leukemic cells, which is in accordance with previously published data.\(^15\)

Currently existing models of cancer progression have highlighted both the amplification and/or activation of major oncogenes in promoting the progressive accumulation of genetic abnormalities and the inactivation of specific tumour suppressor genes. Nevertheless, in terms of the variety of detected allelic imbalances and target genes shown to be altered in the literature, the current authors suggest that no obligatory candidate gene or pathway is accountable for causing the transformation of chronic MPN to blast phase. As de novo AML, MPN blast phase appears to be a heterogeneous disease that is likely to have evolved multiple mechanisms to provide a proliferative advantage to the abnormal leukemic clone. Despite the groundbreaking discovery of the JAK2 or MPL mutations in the majority of patients with MPN, it is becoming increasingly evident that these aberrations do not signify either disease-initiating or leukaemia-promoting events. Besides mutations in TET2 and/or ASXL1 that probably affect the epigenetic landscape in MPN cells, the transforming clone may have to accumulate additional secondary transforming events, including genes such as IDH1/2, ETV6, TP53, RUNX1, RAS and/or c-MYC, as well as chromosomal aberrations. The advances of genetic/epigenetic platforms to study paired MPN samples in chronic phase versus blast phase will identify novel somatic alterations and thereby increase understanding of the leukemic transformation of Philadelphia-negative MPN.\(^16\)

Haematological Malignancies

(24F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders, Blood, 2006;107(9): 3676-82.


51. Tefferi A, Laszlo TL, Abdel-Wahab O, et al., IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrand- or blast-phase essential thrombocythemia, polyethylen vera or myelofibrosis, Leukemia, 2010;24(7): 1830–42.


