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

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## 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 *JAK2*V617F 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 *JAK2*V617F, or rare variants, as well as *MPL*515.[1,2] These different mutant alleles all result in a gain of function due to the constitutive activation of tyrosine kinase-dependent cellular signalling pathways.[2–4] Recently discovered deletions or mutations involving *TET2*, *ASXL1* and *IDH1/2* shed more light on possible mechanisms of excessive proliferation in chronic MPN.[5–7] 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.[8–12]

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,[12] whereas leukaemic evolution occurs in 2–5% of these patients with either PV or ET[8,9] and roughly 20% of cases with PMF.[10,11] 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.[11,13–15] 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 *JAK2*V617F is associated with an increased hyperproliferative disease and may be important for disease progression, at least from PV to secondary myelofibrosis.[16] Moreover, one longitudinal prospective study suggested that the presence of a *JAK2*V617F haematopoietic clone was significantly associated with leukaemic transformation in PMF.[17] The role of *JAK2*V617F 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 *JAK2*V617F

compared with patients with heterozygous *JAK2*V617F or wild-type *JAK2* genes.[18] No disease progression was observed between the *JAK2*V617F(-) chronic phase and *JAK2*V617F(+) blast phase of MPN. Additionally, several other groups have reported a decrease in allelic burden or even a complete disappearance of *JAK2*V617F at the time of leukaemic transformation.[19–21] 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 *JAK2*V617F became negative for these abnormalities in the blast phase.[18] Taken together, the initial aberrant clone of MPN cells may be *JAK2*V617F(-) with a higher propensity to undergo clonal evolution, whereas this evolution particularly favours the clone acquiring the *JAK2*V617F during the chronic phase of MPN. Therefore, the presence of this mutation does not appear to be a prerequisite for the leukaemic transition of MPN, suggesting that additional genetic events are required for full transformation.

One of the molecular mechanisms associated with leukaemic 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 leukaemic 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. $^{32}$P). Both therapies are known to cause DNA damage together with a significant rate of *TP53* aberrations.[24] It was also noted in patients who underwent leukaemic evolution that either loss of heterozygosity (LOH) at 17p or mutation of *TP53* was related to prior exposure to hydroxyurea, a non-alkylating agent.[25,26] However, large prospective studies of MPN patients concluded that the leukaemogenic potential of this cytoreductive compound by itself is low, whereas the risk increases when hydroxyurea is combined with other chemotherapeutic drugs known to damage DNA.[7,27,28] 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 *de novo* AML.[30] They also occur in chronic myelomonocytic leukaemia (CMML) and juvenile myelomonocytic leukaemia (JMML),[31] two chronic myeloid diseases close to but separate from MPN. Mutations in *RAS* have also been reported in some cases during the chronic phase of PMF, but not in PV or ET.[32,33] Although oncogenic *RAS* expression is associated with pronounced proliferation in myeloid lineage cells, transition from MPN to AML is only rarely associated with a *RAS* mutation.[22,23,32–34]

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 leukaemic transformation.[18] Confirming the hypothesis that evolution from MPN to leukaemia 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 *JAK2*V617F. 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.[35–38] 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.[26,34] Notably, transduction of an expression vector carrying mutant *RUNX1* into CD34+ cells from MPN patients markedly promoted their proliferation, independently of the presence of *JAK2*V617F.[34] Moreover, our group found gain of chromosomal material at 8q24.21 (*c-MYC*), including trisomy 8, almost exclusively in the *JAK2*V617F(-) transformed samples.[18] This suggests that the increased oncogenic activity of c-MYC is associated with a strong selection of leukaemic clones that can almost completely displace the myeloproliferative compartment with the *JAK2* gain-of-function mutation.[18]

In addition to aberrations involving known oncogenes (*c-MYC*) or tumour suppressor genes (*ETV6*, *TP53*, *RUNX1*) with already-established leukaemogenic 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.[18] 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.[39–41] 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,[42] and the *CUTL1* gene encodes for a *CUT* family member of the homeodomain proteins that can repress the expression of developmentally regulated myeloid genes.[43] Moreover, genome-wide inspection for minimal regions of duplications/amplifications revealed several interesting genes including *PIN1*, *ICAM1* and *CDC37* 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,[44–46] *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.[47] 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.[5,48] *TET2* mutational frequencies did not differ across the MPN subcategories (13–17%) and appeared independently of *JAK2*V617F. Comparable to family member *TET1*, *TET2* inactivation may have epigenetic consequences that deregulate genes involved in early haematopoiesis as well as in myeloid differentiation.[49] 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 leukaemic transformation.[48] The *ASXL1* gene is mutated in approximately 20% of chronic- or blast-phase MPN cases. Analysis of serial samples from patients with MPN that evolved into AML demonstrated that these mutations were always present during the chronic phase of the

disease.[6,50] *ASXL1* belongs to a three-member family of trithorax and polycomb protein enhancers, which are generally involved in the modulation of development-related genes through chromatin remodelling. The haploin sufficiency of *ASXL1* may have a role in the pathogenesis of MPN and other myeloid malignancies, but did not appear to be acquired during leukaemic transformation.[6]

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.[7] 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.[51] By contrast, the *IDH* mutational rate was significantly more prevalent in the blast phase of MPN, at 21.6%, independent of other known mutations including *JAK2*, *MPL* and *TET2*. Interestingly, the presence of an *IDH* mutation in MPN blast-phase patients also predicted a worse survival, which strengthens the suggestion of its essential pathogenetic contribution to leukaemic transformation. However, functional studies are needed to define the exact role of *TET2*, *ASXL1* and *IDH1/2* in normal and malignant haematopoiesis, and specifically in the transformation from MPN to AML.

Mutations of the *c-CBL* gene in myeloid disorders are strongly associated with LOH on 11q, and are commonly diagnosed in patients with JMML and CMML.[52] However, although MPN shares some clinical as well as haematological features with these entities, *c-CBL* mutations and/or 11q LOH are infrequent in MPN samples of either chronic phase or blast phase, suggesting that *c-CBL* plays only a minor role in the direct process of leukaemic transformation of MPN.[18,26,53]

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.[36] Recently, copy-number neutral (CNN)-LOH has been frequently detected in myeloid malignancies. It is defined as the presence of a chromosome 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 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.[54–57] 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*.[18] In contrast to LOH on 17p, the prognostic impact of 9p CNN-LOH was independent of established risk factors such as -7/7q- or complex karyotype. 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 7q showed poor survival, since this is known to be predictive for rapid progression and reduced response in AML therapy.[39–41] Interestingly, MPN blast-phase patients with 7q CNN-LOH had comparable survival rates to those with -7/7q- in their leukaemic cells, which is in accordance with previously published data.[58]

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 leukaemic 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 leukaemic transformation of Philadelphia-chromosome-negative MPN. ∎

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