Histological and Molecular Classification of Chronic Myeloproliferative Disorders in the Age of JAK2: Persistence of Old Questions despite New Answers

Kais Hussein Oliver Bock Hans Kreipe

Institute of Pathology, Hannover Medical School, Hannover, Germany

Key Words

Chronic myeloproliferative disorders • Classification • Janus kinase 2

Abstract

The discovery of the Janus kinase 2 gain-of-function V617F mutation (JAK2^{V617F}) provided a major breakthrough in the understanding of Philadelphia chromosome-negative chronic myeloproliferative disorders (Ph⁻ CMPD). Among haematologic neoplasm the mutation appears to be almost specific for Ph⁻ CMPD but the different entities comprising polycythaemia vera (PV), essential thrombocythaemia and chronic idiopathic myelofibrosis (CIMF) are not discriminated by the mutation. It is unclear how the diversity with heterogeneous clinical and pathoanatomical presentations comes about. It has been suggested that differences in JAK2^{V617F} gene dosage or different degrees to which the haematologic lineages are affected by the mutation could explain the heterogeneity of morphology and prognosis. Indeed the mutation mediates a PV-like phenotype but with regard to myelofibrosis JAK2^{V617F} does not appear to be a causative factor. Megakaryocytes are homozygous in the majority of fibrotic CIMF and PV, whereas JAK2^{V617F} heterozygosity is predominantly encountered in prefibrotic CIMF and essential thrombocythaemia but transition from hetero- to homozygosity with onset of fibrosis is rare. In conclu-

sion, JAK2^{V617F} provides a valuable adjunct to the diagnosis of Ph⁻ CMPD, in particular with regard to discrimination from reactive proliferations, but the challenge of correct subtyping and hence prognostication persists for clinicians and bone marrow pathologists.

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Introduction

Chronic myeloproliferative disorders (CMPD) represent clonal proliferations of a pathological haematopoietic stem cell (HSC) and affect all or a subset of the haematopoietic lineages, resulting in a mono-, bi- or trilinear proliferation of megakaryocytic, erythroid and granulocytic precursor cells. CMPD encompass chronic myeloid leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET) and chronic idiopathic myelofibrosis (CIMF; synonymously also chronic megakaryocytic granulocytic myelosis, myelofibrosis with myeloid metaplasia or agnogenic myeloid metaplasia). Chronic neutrophilic leukaemia and chronic eosinophilic leukaemia represent rare forms of CMPD [1].

Recognized at an early date [2], the CMPD were grouped together as a closely related set of haematologic disorders by Dameshek [3, 4] but the pathogenesis remained a mystery. Despite overlap in clinical presenta-

tion, there are distinct morphological features which enable subtyping of CMPD in most instances. CMPD entities are characterized by altered bone marrow histology with a mono- to trilinear sustained proliferation and cellular atypia, the latter predominantly affecting megakaryocytes. Facultative changes are marrow fibrosis, blast increase and cytogenetic abnormalities. Clinical criteria used for subtyping by the Polycythemia Vera Study Group [5] comprise enhanced blood cell counts, anaemia and organomegaly of the liver and spleen induced by extramedullary haematopoiesis. Complications may be caused by thrombotic events, haemorrhagic diatheses and constitutional weakness, and major inauspicious prognostic factors are blastic transformation into acute myeloid leukaemia (AML), severe myelofibrosis, portal hypertension and infection.

Challenges in Subtyping of Philadelphia Chromosome-Negative CMPD (PV, ET and CIMF)

Typical bone marrow histology in PV displays prominent proliferation of all haematopoietic cell lineages with increase in erythropoiesis as well as granulopoiesis and almost complete erasure of fatty tissue. In early stages, the hypercellularity may not be as prominent with a considerable amount of preserved fat cells and a predominant megakaryocytic proliferation. In these cases discrimination from ET may pose a severe problem which can only be resolved by follow-up observation [6]. In the so-called spend phase or post-PV myelofibrosis with progressed bone marrow fibrosis, distinction from fibrotic CIMF may be impossible on morphological grounds alone.

Another challenge encountered when classifying CMPD is the prediction of fibrotic progression in thrombocytotic proliferations. A thrombocytosis without fibrotic risk is defined as ET and characterized by loose clusters of enlarged to giant megakaryocytes with hyperploid nuclei, but no increase in cellularity with normal erythropoiesis and granulopoiesis, and no reticulin fibrosis [1]. Cellular CIMF, which by contrast carries a higher risk of evolution to myelofibrosis, is indistinguishable on clinical grounds from ET. This type of ET-like thrombocytosis is considered as an early stage of CIMF and hence categorized as prefibrotic or cellular CIMF because both disorders share atypical giant to medium-sized megakaryocytes with bulbous, cloud-like nuclei, and follow-up studies provided evidence for the progression of cellular to fibrotic CIMF over time [7]. In a

considerable proportion of cases, clear-cut differentiation of ET from prefibrotic CIMF may be very difficult even for experienced bone marrow histopathologists. Furthermore, prefibrotic CIMF cases exhibit tremendous variability with regard to the duration of the stable phase before progression to fibrosis occurs, ranging from a few months to decades.

Molecular Markers for CMPD

Recent years have brought about considerable progress in our understanding of the molecular events which give rise to CMPD. From these insights valuable markers have emerged which provide an indispensable adjunct in diagnosis finding. Almost 20 years ago a CML-specific molecular alteration was identified, characterized by the expression of the chimeric fusion protein BCR/ABL which results from the Philadelphia chromosome translocation t(9;22). Since that time CMPD have been subdivided into t(9;22)(q34;q11) BCR/ABL fusion gene/Philadelphia chromosome-positive (Ph⁺) [8, 9] and Philadelphia chromosome-negative (Ph⁻) disorders. Another milestone was achieved with the most recently discovered 1849G>T mutation in exon 12 of the Janus tyrosine kinase (JAK) 2 gene [10–17]. This hot spot point mutation is located on chromosome 9p and leads to a gain of function due to a defective valine-to-phenylalanine exchange at the amino acid position 617 (V617F) of the autoinhibitory JH2 pseudokinase domain of JAK2. Thus the mutated JAK2^{V617F} tyrosine kinase is constitutively activated and conveys erythropoietin (EPO) hypersensitivity and growth factor independence to transfected cell lines. After antecedent irradiation, transplantation of JAK2^{V617F}-transfected cells was sufficient to mimic human PV [10, 18, 19] and its evolution towards myelofibrosis [18, 19] in murine in vivo models. Interestingly, already in 1997 a gain of function point mutation of the *Drosophila melanogaster* JH2 homologue was linked to a leukaemia-like aberrant haematopoiesis [20] and in 1999 an unsuccessful systematic search for a putative alteration in the JH2 domain of JAK2 was performed in patients suffering from myelodysplastic syndromes (MDS), CML, AML and acute lymphoblastic leukaemia, but not Ph⁻ CMPD [21].

In a systematic study, a panel of 79 cell lines representing CMPD, MDS, AML and acute lymphoblastic leukaemia was analysed for the JAK2 status, and CMPD/MDS-derived MB-02, MUTZ-8, UKE-1 and SET-2 as well as erythroleukaemia-derived HEL were positive for the mu-

tation. Among them, only SET-2 proved to be heterozygous for the mutation, while the other 4 cell lines displayed homozygosity [22]. In clinical samples, Ph⁻ CMPD were affected by the mutation, whereas other myeloid malignancies only exceptionally revealed the mutation [16, 17, 23–28] and Ph⁺ CML, lymphoid malignancies and non-neoplastic haematopoiesis lack the JAK2 mutation completely [17, 29, 31].

The gain of function mutation in JAK2^{V617F} affects the JAK/STAT cellular signal transduction pathway. Via cytokine-mediated receptor stimulation, signal transducers and activators of transcription (STAT) are activated through JAK-mediated tyrosine phosphorylation. This leads to STAT dimerization, nuclear translocation and regulation of target gene expression by several downstream effectors.

The aberrant JAK2^{V617F}/STAT5 signal cascade is hypersensitive to EPO [10, 29], thrombopoietin (TPO), interleukin-3 [15] and granulocyte colony-stimulating factor (G-CSF) [32]. In the myeloid lineages, the gain of function is essentially dependent on related type I cytokine receptors for EPO, G-CSF and TPO [33]. Paradoxically, some cytokines may also have inhibitory effects for yet unknown reasons. In vitro culturing of CD34⁺ cells derived from peripheral blood of mutated PV patients showed that increased EPO levels resulted in decreased survival of the JAK2^{V617F} clone [34]. Compensatory negative regulating feedback mechanisms of JAK/STAT, particularly suppressors of cytokine signalling (SOCS), may react to this EPO hyperstimulation and constitutively activated JAK2^{V617F}. While this particular question is largely unsettled, it has been demonstrated that G-CSF stimulation in JAK2^{V617F} CMPD patients did not have any significant influence on SOCS-3 expression [32]. Referring to this, SOCS-1 is overexpressed in PV, CIMF and ET but did not correlate with the underlying JAK2 mutation, probably reflecting a JAK2^{V617F}-independent general negative feedback mechanism against autonomous proliferation in all Ph⁻ CMPD subtypes [35]. Furthermore, we investigated Friend leukaemia integration-1 (Fli-1) expression, which is involved in lineage commitment of megakaryocytes and myeloid progenitors through induction of JAK, and we could demonstrate that Fli-1 expression levels were significantly elevated in JAK2^{V617F} ET [36]. However, in other Ph⁻ CMPD entities the mutation status per se showed no influence on the Fli-1 expression [36]. These findings indicate the absence of a simple up and down regulatory mechanism and suggest a hidden complex control cascade.

In the first descriptions of the JAK2^{V617F} mutation, it was believed that only the myeloid lineage was affected [10–17, 29, 37], although lymphoid involvement was not precluded [9]. Recent findings in PV and CIMF indicate that in fact the mutation is acquired at the putative earliest level, namely in the pluripotent HSC [34, 38, 39]. In order to characterize the HSC in JAK2^{V617F}-mutated PV, CD34-positive HSC were selected and expanded in an in vitro differentiation assay. Differentiating HSC did not transmit the mutation to all haematopoietic lineages. Whereas granulocytes and megakaryocytes/platelets carried the molecular defect, precursor B and T cells and mature CD19⁺ B and CD3⁺ T lymphocytes were affected only in a minor subset of PV and CIMF samples, or solely B lymphocytes belonged to the JAK2^{V617F} clone [34, 39]. Interestingly, the frequency of mutated JAK2 allele was observed to be 3 times lower in B cells and about 12 times lower in T cells than in CD34⁺ and myeloid cells [34], indicating a proliferation disadvantage for the JAK2^{V617F} lymphoid cells. Thus it is most likely that the mutation affects an aberrant HSC, but with regard to the absent or very low allele frequency in lymphocytes, only a minority of lymphoid precursor cells reach maturation. Accordingly, lymphocytes do not express the adequate receptors providing the first link in the chain between JAK2^{V617F}/STAT and downstream intracellular signal transduction [32]. Referring to this, the clinical relevance of lymphocytic clonal involvement remains doubtful and until now no JAK2^{V617F}-genuine lymphoid malignancy [15–17] has been reported. Even in a case of pre-existing JAK2^{V617F} PV a succeeding lymphoid neoplasm did not originate from the same clone [40].

In rare cases the mutation might possibly be acquired at different levels other than HSC, after the segregation of the myeloid lineages. It has been shown that in PV solely the erythroid lineage can harbour the mutation [41] while in ET predominantly megakaryocytes can be affected [42].

Very recently, a systematic search for genetic aberrations in haematopoietic-specific cytokine receptors in Ph⁻ CMPD revealed in about 5–9% of ET and CIMF patients another gain of function point mutation in the thrombopoietin receptor (MPL^{W515L} or MPL^{W515K}) [43, 44]. Interestingly, most of the MPL-mutated cases had a JAK2 wild-type status, but a few cases carried MPL^{W515L} and JAK2^{V617F} concurrently [44]. The relevance for diagnostic purposes particularly in JAK2 wild-type Ph⁻ CMPD and therapeutic consequences remain to be further evaluated.

Methods for the Analysis of JAK2^{V617F}

Usually, the detection of JAK2 mutation is technically undemanding and reliable, but the costs of devices and methods range from relatively cheap to expensive. For diagnostic and research purposes, DNA and RNA/cDNA can be obtained from peripheral blood (mononuclear cell fraction, granulocytes, platelets and flow-cytometrical fractionated cells), formalin-fixed and paraffin-embedded bone marrow trephines or bone marrow-derived laser microdissected cells, such as megakaryocytes [10–17, 30, 31, 42]. Even solid organ tissues, such as liver biopsies showing extramedullar haematopoiesis, are suitable for JAK2 detection (own unpublished data).

The JAK2 status can be expressed in different ways, either simply as mutated/unmutated, as wild-type/heterozygous/homozygous or as the gene dosage/allele frequency of the percentage of mutated G>T allele. It should be taken into account that the JAK2 status can be changed by intermingled stromal cells, e.g. bone marrow fibrocytes, and/or other polyclonal JAK2 wild-type cells, such as lymphocytes. Different methods can be used to detect the 1849G>T transition in DNA and/or cDNA. A well known and simple to use detection method for single nucleotide polymorphisms (SNP) are restriction enzymes. The *BsaX* I restriction enzyme is suitable for digestion of the wild-type JAK2 polymerase chain reaction (PCR) product, but fails to recognize the mutant G>T hot spot, leaving the PCR product unfragmented [11, 17]. Amplification refractory mutation system (ARMS) assay is based on a tetra-primer JAK2-PCR, consisting of 2 primer pairs for the simultaneous amplification of the wild-type and mutated SNP alleles [11]. Both assays have the advantage of employing only standard PCR devices and some specific reagents. Pyrosequencing® is based on a luciferase-dependent light signal, which is proportional to pyrophosphate release for every incorporated nucleotide at the SNP hot spot. Apart from pyrosequencing [16], real-time PCR-based assays [17, 29, 45] and capillary electrophoresis [10, 14] also allow a quantification of the ratio of wild-type G to mutant T allele. Of note, primer sequences and product lengths may vary between different publications without affecting the overall result. Actually, a systematic comparison of all available methods has not been done so far, but for validation reasons the generated JAK2-PCR products are often used for more than one method. For example, ARMS assay and *BsaX* I restriction analysis can be combined if requested [11].

Interestingly, we (unpublished data) and others [12, 34] observed that in the same sample the percentage of

mutated T allele is constantly higher in cDNA than genomic DNA. Whether the mutant allele might have a transcriptional advantage or whether JAK2-mutated mRNA is more stable than the wild-type form remains to be resolved. However, this implies the use of cDNA for diagnostic purpose, which might be more sensitive.

Detection of JAK2^{V617F} in Bone Marrow Trephines

Unlike the BCR/ABL junction, the JAK2^{V617F} abnormality in CMPD exhibits 2 peculiar features: (1) it is not specific for one type of CMPD but occurs at different frequencies in all Ph⁻ CMPD and (2) although Ph⁻ CMPD provide clonal proliferations, the JAK2^{V617F} aberration is neither present in all non-lymphoid lineages and nor in all cells of one identical lineage.

With regard to the first issue, a number of studies have shown that all Ph⁻ CMPD subtypes may be affected by mutated JAK2^{V617F}, the highest rate occurring in PV (65–97%; 21–70% homozygous), whereas variable proportions were found in CIMF (35–95%, 9–22% homozygous) and ET (23–75%, 0–6% homozygous) [10–17, 23–31, 46–48, 50]. For a detailed summary of mutation frequencies in different myeloid and lymphoid entities see table 1. Because the variability of findings could be due to different classification schemes of CMPD, we performed an investigation on more than 340 bone marrow trephines exhibiting typical CMPD classified according to the WHO, including 79 previously published cases [31]. In this ongoing study more than 95% of PV were positive whereas ET and CIMF revealed similar rates of mutation of about 60%. No differences could be found between prefibrotic and manifest CIMF.

The high rate of JAK2^{V617F} and the high frequency of homozygous JAK2 status in PV give rise to the question whether this mutation represents a key event in PV genesis. In fact, JAK2^{V617F} is sufficient to mediate a PV-like phenotype in mouse models [10, 18, 19]. In ET patients, the presence of JAK2^{V617F} is associated with elevated levels of haemoglobin and haematocrit as well as increased bone marrow erythropoiesis and granulopoiesis [47–50]. Additionally, a small subset of JAK2^{V617F} ET patients even transform into manifest PV [47]. Consequently, it has been suggested that CMPD are better characterized as a phenotypical continuum with emphasis on different features and that JAK2 mutation status modulates rather than strictly separates entities [49]. With regard to the second aforementioned peculiarity, another surprising feature was observed in female cases of ET which were

Table 1. Mutation frequencies in different myeloid and lymphoid entities

	JAK2 ^{V617F} /n	Mutation frequency	Reference	Summation of all data
<i>Myeloid disorders</i>				
PV	60/62 71/73 27/28 58/63 26/29 40/45 25/29 58/72 121/164 83/128	97% (70% +/+) 97% 96% 92% (21% +/+) 90% 89% 86% 81% 74% (25% +/+) 65% (27% +/+)	Lippert et al. [46] Baxter et al. [13] Horn et al. [30] Tefferi et al. [47] Bock et al. [31] James et al. [10] Jelinek et al. [17] Jones et al. [15] Levine et al. [16] Kralovics et al. [14]	569/693 (82%)
CIMF	18/19 28/45 13/23 16/28 8/16 15/35 3/7 16/46	95% 62% 57% (22% +/+) 57% 50% 43% 43% 35% (9% +/+)	Jelinek et al. [17] Horn et al. [30] Kralovics et al. [14] Bock et al. [31] Baxter et al. [13] Jones et al. [15] James et al. [10] Levine et al. [16]	117/219 (53%)
ET	45/60 17/23 29/51 414/806 73/130 73/150 9/21 24/59 37/115 3/10 4/15 21/93	75% (0% +/+) 74% 57% 53% 56% (6% +/+) 49% 43% 41% 32% (3% +/+) 30% 27% 23% (3% +/+)	Lippert et al. [46] Horn et al., [30] Baxter et al. [13] Campbell et al. [11] Antonioni et al. [48] Wolanskyj et al. [50] James et al. [10] Jones et al. [15] Levine et al. [16] Jelinek et al. [17] Bock et al. [31] Kralovics et al. [14]	749/1,533 (49%)
Ph ⁺ CML	0/99 0/5 0/4	0% 0% 0%	Jelinek et al. [17] Bock et al. [31] Horn et al. [30]	0/108 (0%)
Ph ⁻ CML	3/16	19%	Jelinek et al. [17]	
Chronic neutrophilic leukaemia	1/6 case report (JAK2 ^{V617F}) case report (JAK2 ^{V617F})	17%	Steensma et al. [23] Mc Lornan et al. [24] Lea et al. [25]	3/8 (38%)
CMPD UC	13/52 6/8	25% 75%	Baxter et al. [13] Horn et al. [30]	19/60 (32%)
<i>De novo</i> AML	1/93 0/28 0/10	1% 0% 0%	Levine et al. [16] Jelinek et al. [17] Bock et al. [31]	1/131 (<1%)
Acute megakaryoblastic leukaemia	2/11	18%	Jelinek et al. [17]	
AML from pre-existing CMPD	3 case reports (JAK2 ^{V617F})		Levine et al. [16]	

Table 1 (continued)

	JAK2 ^{V617F} /n	Mutation frequency	Reference	Summation of all data
MDS	5/101 2/38 2/48 1/68	5% 5% 4% 2%	Steensma et al. [23] Ohyashiki et al. [26] Levine et al. [16] Jelinek et al. [17]	10/255 (4%)
Chronic myelomonocytic leukaemia	7/52 3/119 1/47	14% 3% 2%	Jelinek et al. [17] Steensma et al. [23] Johan et al. [27]	11/218 (5%)
Juvenile myelomonocytic leukaemia	case report (JAK2 ^{V617F})		Tono et al. [28]	
Systemic mastocytosis	2/8 0/28	25% 0%	Steensma et al. [23] Jones et al. [15]	2/36 (6%)
<i>Lymphoid disorders</i>				
B cell acute lymphoblastic leukaemia	0/83 0/20 0/10	0% 0% 0%	Levine et al. [29] Jelinek et al. [17] Bock et al. [31]	0/113 (0%)
T cell acute lymphoblastic leukaemia	0/93	0%	Levine et al. [29]	
B cell chronic lymphocytic leukaemia	0/45	0%	Levine et al. [29]	

concomitantly studied for X-chromosome inactivation patterns and JAK2^{V617F}. They displayed both the JAK2^{V617F} mutation and polyclonality of granulocytes and platelets [51]. Obviously in these cases the JAK2^{V617F} clone did not have the potency to overgrow polyclonal haematopoiesis. Similar observations were made by Kralovics et al. [52] who found that the percentage of granulocytes and platelets with JAK2^{V617F} was often markedly lower than the percentage of clonal granulocytes determined by IDS or MPP1 clonality assays. The presence of JAK2 in only a subfraction of clonal cells was confirmed in 2 samples with 20q deletions as an autosomal, X-chromosome-independent clonality marker. From these results it was concluded that in a proportion of patients with CMPD, JAK2^{V617F} occurs on the background of clonal haematopoiesis caused by a somatic mutation in an as-yet-unknown gene [52]. Very low JAK2^{V617F} allele frequencies of <10% are possible [34], but the biological significance remains elusive.

From these findings it emerges that diversity of Ph⁻ and JAK2^{V617F}-mutated CMPD could be either due to a combination with other clonal and more specific defects or to different gene dosages and lineage involvement by JAK2^{V617F}. In order to investigate the latter hypotheses we conducted a study on allele frequency in bone marrow

trephines from CMPD using pyrosequencing. The majority of PV and fibrotic CIMF exhibited allele frequencies of more than 50%, suggesting homozygosity. In ET and pre-fibrotic CIMF, most cases exhibited heterozygosity with allele frequencies below 50%. Because gene dosages may also be influenced by the rate of transcription, we quantified JAK2^{V617F} transcripts in ET and pre-fibrotic CIMF. Although the percentage of mutant T allele was constantly higher in cDNA than DNA, no differences could be noted between the 2 entities (unpublished data).

For the investigation of lineage involvement we purified megakaryocytes from bone marrow sections because atypical megakaryocytes provide a hallmark of most CMPD. The allele frequency in purified megakaryocytes was compared to that in the residual bone marrow purified from megakaryocytes [42]. With regard to homo- and heterozygosity, similar results were obtained in purified megakaryocytes compared to the complete cellularity with the exception of ET. In ~25% of the ET cases the JAK2^{V617F} allele frequency in isolated megakaryocytes exceeded that of the non-megakaryocytic bone marrow cells by more than 2-fold [42]. In about a quarter of the ET cases more than 50% of the megakaryocytic alleles were affected corresponding to homozygosity at least in a subfraction of the megakaryocytes [42].

Another question is the relationship of JAK2^{V617F} to fibrotic progression. In isolated megakaryocytes from manifest CIMF, more homozygous mutations were found than in cells isolated from prefibrotic cases (60% vs. 33%) [42] and homozygosity is associated with a higher risk of fibrotic transformation in PV [46]. Sequential biopsies derived from cases with fibrotic progression of cellular CIMF, however, did not show an association between gene dosage and onset of fibrosis [42]. Furthermore, among more than 80 CIMF cases with sequential biopsies not a single case was encountered with newly occurring JAK2^{V617F} (own unpublished data) which could have been expected according to the findings of Kralovics et al. [52]. Because the JAK/STAT signalling pathway is involved in the regulation of genes encoding matrix metalloproteinases (MMP), we examined the expression of MMP, their tissue inhibitors and collagen types in relation to the JAK2 status (V617F mutation versus wild-type) in CIMF. Whereas no correlation was found between the JAK2 mutation status and MMP gene products, there was an evident association of the latter with the stage of disease [52].

Whereas JAK2 analysis appears to be of little help in the classification of Ph⁻ CMPD and prediction of fibrotic progression, an important application in routine diagnosis is obviously the verification of a neoplastic haematological disorder and discrimination from reactive erythrocytosis or thrombocytosis. Analysis can be available within few days and has proved to be very helpful e.g. in Budd-Chiari syndrome [54, 55]. Furthermore, the dogma that elevated serum EPO levels in Budd-Chiari syndrome patients exclude the diagnosis of PV is no longer supportable, since in a proportion of these patients the JAK2 mutation was detectable [55].

The JAK2 status in CMPD patients interferes, if not with the histopathological manifestation, with the clinical course; more complications have been implied in JAK2^{V617F} cases, such as secondary fibrosis, haemorrhage and thrombosis, and these patients have received cytoreductive treatment more often than JAK2 wild-type patients [15]. Homozygously mutated PV patients revealed higher levels of haemoglobin and greater risk of fibrotic transformation than heterozygous patients [47]. CIMF patients harbouring the mutation were demonstrated to have shorter survival [56], and JAK2^{V617F} ET cases showed higher tendency for polycythemia, thrombosis, leukocytosis and advanced age at diagnosis [48, 50, 57]. With regard to chemosensitivity, JAK2^{V617F} ET patients seemed to have more benefit from hydroxyurea treatment than wild-type JAK2 patients, whereas such an effect was not

obvious in patients receiving anagrelide [49]. This issue is of potential interest and needs more randomized trials including JAK2 mutation status as one of the study criteria. Moreover, strictly applied WHO criteria for classification of Ph⁻ CMPD subtypes, along with the most reliable tests for detection of a potentially underlying JAK2 mutation, are indispensable for a correct estimation of response rates in studies conducted to determine the efficacy of therapeutic regimes.

It was questionable which role the JAK2 mutation might play in the worst biological end-stage of CMPD, namely the transformation into acute leukaemia [15]. Therefore, 42 CIMF patients with leukaemic transformation were analysed for JAK2 mutation status and prognostic correlations, but besides younger age and shorter interval to leukaemic transformation no differences in survival were observed [58]. However, more future studies are required to verify the role of JAK2 status as a prognostic marker.

Summary

The discovery of the JAK2^{V617F} mutation in the majority of Ph⁻ CMPD is comparable to the breakthrough enabled by the detection of the BCR/ABL fusion gene. Since its discovery in 2005 the new molecular marker rapidly found its way into clinical diagnostics. Whereas it is very useful in discriminating neoplastic from reactive myeloproliferations, it appears to be of little help in subtyping of CMPD and prediction of fibrotic progression. The JAK2^{V617F} abnormality most likely acts in concert with other clonal and yet unknown molecular defects, and modifies the clinical course rather than determines the histopathological subtype of Ph⁻ CMPD. Higher proportions of homozygously mutated cases are found in PV and fibrotic CIMF than in ET and prefibrotic CIMF, but in individual follow-up studies fibrotic progression could not be linked to an increase in gene dosage. In ET the megakaryocytic lineage can be selectively or predominantly affected by the mutation.

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