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Comparison of mutated *JAK2* and *ABL1* as oncogenes and drug targets in myeloproliferative disorders

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Abstract

Constitutively activated mutants of the non-receptor tyrosine kinases (TK) ABL1 and JAK2 play a central role in the pathogenesis of clinically and morphologically distinct chronic myeloproliferative disorders but are also found in some cases of *de novo* acute leukemia and lymphoma. Ligand-independent activation occurs as a consequence of point mutations or insertions/deletions within functionally relevant regulatory domains (JAK2), or the creation of TK fusion proteins by balanced reciprocal translocations, insertions or episomal amplification (ABL1 and JAK2). Specific abnormalities are correlated with clinical phenotype, although some are broad and encompass several WHO-defined entities. TKs are excellent drug targets as exemplified by the activity of imatinib in *BCR-ABL1*-positive disease, particularly chronic myeloid leukemia. Resistance to imatinib is seen in a minority of cases and is often associated with the appearance of secondary point mutations within the TK domain of BCR-ABL1. These mutations are highly variable in their sensitivity to increased doses of imatinib or alternative TK-inhibitors such as nilotinib or dasatinib. Selective and non-selective inhibitors of JAK2 are currently being developed and encouraging data from pre-clinical experiments and initial phase-I-studies regarding efficacy and potential toxicity of these compounds have already been reported.

Keywords

chronic myeloproliferative disorders; tyrosine kinase; *ABL1*; *JAK2*

Introduction

In 1951, William Dameshek recognized the clinical and pathologic overlaps and similarities between the entities now referred to as, chronic myeloid leukemia (CML), polycythemia vera (PV), primary myelofibrosis (PMF) and DiGuglielmo's syndrome, and grouped them under a single category called chronic myeloproliferative disorders (MPDs) (1). MPDs are now considered to include essential thrombocythemia (ET) and also several much rarer entities, some of which also show dysplastic features (2). Already in 1951, Dameshek considered the possibility that these conditions might represent variable phenotypes caused by a yet unknown stimulus leading to uncontrolled proliferation of hematopoietic cells (see

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review (3)). Although the puzzle of the pathogenesis of MPDs has not been completely resolved more than 50 years later, it has become evident that constitutive activation of tyrosine kinases (TKs) or TK dependent pathways represents the pathogenetic hallmark of these diseases.

A major breakthrough in this field was achieved in 1960 when Nowell and Hungerford first described a shortened chromosome 22 in CML, the so-called Philadelphia (Ph) chromosome (4). In 1973, Rowley found that this chromosome abnormality is the result of a balanced reciprocal translocation involving the long arms of chromosomes 9 and 22, t(9;22) (5). Further genetic investigations revealed that the *ABL1* gene on chromosome 9 was translocated to chromosome 22 and fused to a new gene termed 'breakpoint cluster region' (*BCR*). The resulting *BCR-ABL1* fusion was shown to result in the production of a constitutively active cytoplasmic TK that does not block differentiation, but enhances proliferation and viability of myeloid lineage cells and is likely sufficient to cause CML by itself (6-9).

The suggestion of wider involvement of TKs in MPDs came from the analysis of rare translocations. More than 40 novel fusion genes involving various TKs such as *PDGFRA* (e.g. *FIP1L1-PDGFRA*), *PDGFRB* (e.g. *ETV6-PDGFRB*), *JAK2* (e.g. *PCM1-JAK2*), *FGFR1* (e.g. *ZNF198-FGFR1*) or *ABL1* (e.g. *ETV6-ABL*) have been identified thus far (10). Intriguingly, the vast majority of these fusions are associated with a significant eosinophilia of the blood and marrow in association with a disease that resembles CML (atypical CML), myeloproliferative subtypes of chronic myelomonocytic leukemia (CMML), hypereosinophilic syndrome (HES)/chronic eosinophilic leukemia (CEL), unclassifiable MPDs or MDS/MPD overlap syndromes. Fusion genes have not been described in classical MPD subtypes such as PV, ET or PMF (10) except for very rare cases with *FOP-FGFR1* who presented with PV but then progressed to a more aggressive phenotype (11). Although the proportion of cases harbouring gene fusions is very small, their identification was important because subsequent *in vitro* and *in vivo* studies clearly demonstrated the central role of constitutive activation of the respective TK in the pathogenesis of these disorders.

In 2005, a single point mutation within the non-receptor TK JAK2 leading to a substitution of valine by phenylalanine at amino acid 617 (JAK2V617F) was reported to be present in more than 95% of patients with PV and approximately 50% of patients with ET or PMF (12-16). In addition, point mutations or insertions/deletions within *JAK2* exon 12 have subsequently been identified in nearly all patients with JAK2V617F-negative PV as well as some individuals with idiopathic erythrocytosis, and substitutions of tryptophan at amino acid 515 of the thrombopoietin receptor MPL (W515L, W515K) have been found in 5-10% of patients with PMF and 1-5% of patients with ET (17-19).

Overall, the various known mutations of *ABL1* and *JAK2* account for a substantial proportion of known molecular aberrations in MPDs. Because treatment with tyrosine kinase inhibitors (TKIs) has so fundamentally changed the clinical course of CML, it is widely hoped that similar efficacy and low toxicity will be achieved with inhibitors of JAK2. In contrast to CML, for which imatinib was the only drug being tested in the clinic for many years, numerous JAK2 inhibitors have rapidly been identified, to a great extent based on the experience gained from the development of TKIs in CML. The rational use of JAK2 inhibitors in a spectrum of disorders with different clinical courses may greatly depend on the balance between toxicity and prognosis.

Tyrosine Kinases

TKs are enzymes that catalyze the transfer of phosphate from ATP to tyrosine residues in their own cytoplasmic domains (trans- or autophosphorylation) and tyrosines of other intracellular proteins (see review (20)). These proteins are vital components of the cell signaling machinery contributing to essential cellular functions such as cell growth, proliferation and differentiation. Within the human genome, there are believed to be approximately 90 TKs in total that can be further subdivided into receptor and non-receptor TKs. In their inactive state, receptor TKs exist as monomeric proteins that are embedded in the cellular plasma membrane. Their general structure includes an extracellular ligand binding domain to which various growth factors bind, a domain that traverses the plasma membrane, a juxtamembrane domain that often harbors negative regulatory elements, and a catalytic TK domain that in some cases is split into two parts. Activation through binding of a cognate ligand induces oligomerization of the receptor, which juxtaposes the catalytic TK domains inducing a conformational change, which partially activates the enzymatic activity. As a direct consequence, a key tyrosine residue in the activation loop is autophosphorylated, which further changes the structure of the kinase domain, increasing enzymatic activity and resulting in phosphorylation of additional tyrosine residues. These phosphorylated tyrosines serve as docking sites for adapter proteins that subsequently recruit other downstream cytoplasmic signaling molecules, which mediate the physiologic effects of kinase activation. Together, ligand-stimulated TKs activate multiple signaling pathways via downstream effectors including those involving JAK-STATs, Ras-Raf-MEK-ERK, PI3-K/PKD1/Akt and PLCγ. The non-receptor TKs lack transmembrane domains and are hence located within the cytosol or nucleus. ABL1 and JAK2 belong to this group and require upstream signals such as ligand-bound cytokine receptors or integrin ligation for their activation. The subcellular localization of ABL1 is the cytoplasm and the nucleus (21) whereas JAK2 is mainly located close to the inner plasma membrane. Reports of JAK2 localization within the nucleus remain controversial (22, 23). The mechanism of activation of multiple signaling pathways is similar to that of receptor TKs and the resulting signals may also impact on proliferation, differentiation and apoptosis.

Normal structure and cellular functions of ABL1 and JAK2 proteins

ABL1

The ABL1 (Abelson murine leukemia viral (v-abl) homolog 1) protein and its close relative ABL2, formerly ARG (Abelson-related gene protein), are ubiquitously expressed TKs that resemble in some ways the family of SRC kinases. The *ABL1* gene is located on chromosome band 9q34 and utilises two alternative promoters and associated first exons resulting in two peptides called 1a and 1b, which differ in their N-terminal amino acid sequence. Simplified, the structure of ABL1 consists of N-terminal SH3 and SH2 domains (which mediate binding to proline-rich and phosphotyrosine protein ligands, respectively), a catalytic TK domain that contains phosphotransferase activity, a DNA-binding domain with yet largely unknown effects, and a C-terminal actin-binding domain. Normal activation of the ABL1 proteins requires additional post-translational modifications as phosphorylation, acetylation and myristoylation.

Based on its subcellular localization, which is markedly influenced by post-translational modifications, ABL1 can execute diverse and even contrary functions: within the cytoplasm, ABL1 binds to the actin cytoskeleton and is involved in cytoskeletal organization, nonerythroid myelopoiesis and regulation of small GTPases (see review (24)). Studies using the ABL1 homolog of Drosophila, d-ABL, suggest a possible role in axonal pathfinding (axogenesis) and neuronal development. Cytoplasmatic expression of ABL1 leads to increased cell proliferation and survival. In response to genotoxic stress such as DNA

damage or the increase of reactive oxygen species, ABL1 is translocated into the nucleus where its activity contributes to induction of apoptosis and inhibition of cell growth (25, 26). Alternative studies suggested that it might act as a promoter as well as a suppressor of cell cycle transition under particular circumstances since ABL1 interacts with numerous binding proteins in a cell cycle-dependent manner (27-30).

Only 75% of *ABL1* knockout mice survive the first week (31), the remaining animals show severe defects in spermatogenesis, thymic and splenic atrophy, susceptibility to infections and reductions of T and B cell lymphocytes and their early precursors in lymphoid organs (32-34). Surprisingly, the myeloerythroid system of these *ABL1* mutants is normal (35, 36).

Regulation of ABL1 activity appears to be particularly complex. In part, ABL1 is autoinhibited in an intramolecular fashion that is similar to SRC kinases, where the SH3 domain is engaged with a single proline residue in the spacer region between the SH2 and TK domains (37). Deletion of the first 80 N-terminal amino acid residues of the 1b isoform of the ABL1 protein results itself in increased enzyme activity, a mechanism that might also contribute to BCR-ABL1 mediated transformation (38). However, there is also considerable evidence that ABL1 is regulated by several cellular inhibitors (24, 39).

JAK2

The JAK proteins (JAnus Kinase or Just Another Kinase) comprise a family of four structural tightly related TKs: JAK1, JAK2, JAK3 and TYK2. This protein family was named after the two-faced Roman god Janus because of their unique carboxy terminal structure consisting of a kinase (JH1) domain and a highly related pseudokinase (JH2 domain) that lacks catalytic activity. Of interest, deletion of the JH2 domain resulted in constitutive activation of the JAK2 TK suggesting that the JH2 pseudokinase domain regulates the JH1 kinase domain in a negative fashion (40). In the zebrafish model, JAK2 is involved in primitive hematopoiesis while deletion of *JAK2* alleles in mice result in embryonic lethality probably due to fatal anemia (41, 42).

The *JAK2* gene is located on chromosome band 9p24 and its activity is required for signaling by several growth factor receptors that lack intrinsic TK activity, such as the erythropoietin receptor (EpoR), the thrombopoietin receptor (Mpl) and the granulocyte colony-stimulating factor receptor (G-CSFR). Binding of ligand leads to receptor dimerization with subsequent conformational changes of the bound JAK2 enzymes on the inner cellular membrane. This requires the integrity of the JAK2 protein-interacting FERM (band four-point-one, ezrin, radixin, and moesin) domain which is located at the N-terminal region of JAK2 (43). Subsequently, JAK2 is phosphorylated (trans- or autophosphorylation) which results in increased activity and phosphorylation of downstream molecules such as the signal transducer and activator of transcription family members (STATs). In response to this stimulus, STATs translocate rapidly into the nucleus where they bind to specific DNA sequences and activate transcription. Downstrean targets of STATs include factors involved in diverse processes such as cell proliferation, differentiation, apoptosis, fetal development, inflammation and immune response (44, 45).

Constitutive activation of different JAKs and STATs are believed to mediate neoplastic transformation and promote abnormal cell proliferation in various malignancies, and it has been suggested that JAK2 may be specifically involved in abnormal cell growth induced by BCR-ABL1 in CML (46). Several lines of evidence suggests that constitutive activation of STAT5 represents a major factor which contributes to the malignant transformation process which has been demonstrated by several studies (47-49). However, the absolute requirement of STAT proteins in these transformation processes has not been completely clarified yet.

Mechanisms of aberrant activation and correlating phenotypes of ABL1 and JAK2

Uncontrolled activation of TKs is considered a driving factor in the pathogenesis of many human malignant disorders. In myeloid leukemias, two major molecular mechanisms have been discovered that interfere with endogenous TK function, leading to constitutive activation and potential malignant growth. The first mechanism involves the formation of a fusion gene between a kinase and an independent, unrelated gene. This fusion gene encodes a chimeric protein that consists of the TK C-terminal region including the entire catalytic domain linked to the N-terminus of the partner gene. The kinase activity of the TK moiety is almost always dependent on specific structural features encoded by the N-terminal partner gene, particularly a dimerization or oligomerization domain. The second mechanism leading to constitutive TK activation is the acquisition of intragenic mutations such as base pair substitutions (point mutations), or insertions/deletions within regions encoding essential functional or regulatory sites. In many cases these genetic alterations are oncogenic, however, some may occur at a later time point during disease progression, as will be addressed later in this review.

TK fusion genes involving ABL1

By far the most frequent and best-studied *ABL1* fusion gene is *BCR-ABL1*, which is predominantly found in patients with CML, and, to a lesser extent in pre-B-ALL and occasionally *de novo* AML. *BCR-ABL1* may exist in several different isoforms but the great majority consist of a variable number of *BCR* exons fused to *ABL1* exon 2 with a small percentage having fusions to *ABL1* exon 3 (see review (50)). The fusion between *BCR* exon 1 (e1) and *ABL1* exon 2 (a2) (e1a2 fusion) encodes a chimeric protein of 190 kDa, referred to as $p190^{BCR\text{-}ABL1}$. It is found in 50-70% of *BCR-ABL1*-positive ALL patients and very rarely in monocytosis-associated CML or de novo AML of M4/M5 phenotype. The e13a2 and e14a2 fusion transcripts (often referred to as b2a2 and b3a2, respectively) encode a $p210^{BCR\text{-}ABL1}$ fusion protein of 210kDa in almost all CML cases and in about 30-50% of *BCR-ABL1*-ALL cases. Interestingly, in *BCR-ABL1*-ALL both transcripts exhibit similar characteristics regarding clinical features, disease prognosis and treatment response. The rare e19a2 fusion transcript encodes for a $p230^{BCR\text{-}ABL1}$ fusion protein of 230kDa. This fusion was associated initially with a distinct phenotype defined by mild neutrophilic leukocytosis, low proportion of myeloid precursors, and absent or minimal splenomegaly (51), however several e19a2 cases have been reported subsequently with apparently typical CML. Rare fusion transcripts worth noting, such as e6a2, e8a2 or e15a2, with or without insertion of intron-derived sequences, have been identified in occasional cases of CML and ALL.

CML is typically characterized by three phases of variable duration starting with chronic phase, followed by progression to an accelerated phase and finally resulting in a blastic phase, a state resembling AML. BCR-ABL1 enhances proliferation and viability of myeloid lineage cells and is most likely sufficient to cause chronic phase CML, but over time, other genetic events are believed to occur that cause disease progression to an acute leukemia state, referred to as blast phase (52). It is important to segregate these late mutations promoting disease progression from other essentially random genetic events that have no apparent influence on pathogenesis and therefore are considered to be ‘bystander’ or ‘passenger’ events.

The BCR-ABL1 oncoprotein has the ability to activate diverse downstream signaling pathways, some of which have been shown to contribute to the malignant transformation process. For example, an important component in the BCR-ABL1 cascade in patients with

CML is the activation of the transcription factor STAT5. It is not completely understood how STAT5 becomes activated in BCR-ABL1 transformed cells. Possible explanations are that STAT5 is directly phosphorylated by BCR-ABL1 or indirectly phosphorylated through either JAK2 or the SRC family hematopoietic cell kinase (Hck) (53, 54). Following phosphorylation, STAT5 rapidly translocates into the nucleus where it upregulates the transcription of several genes, notably those that inhibit apoptosis, most likely through upregulation of the proapoptotic factor Bcl-X_L (55).

The phosphoinositide 3-kinase (PI3K) pathway is constitutively activated by BCR-ABL1 and is required for transformation. The PI3K protein phosphorylates phosphatidylinositol (PI) producing PI-(3,4)-bisphosphate and PI-(3,4,5)-trisphosphate that function as second signaling messengers. PI3K is a pleiotropic regulator as it regulates AKT, which is crucial for cell survival and growth; Rac, important for cell motility and survival; S6kinase, which promotes protein synthesis; and several other proteins. Autophosphorylation of tyrosine 177 (Y177) within BCR-ABL1 leads to activation of PI3K and phosphorylation of an adapter protein known as Gab2, which forms a complex with Grb2 (56). PI3K regulation is strongly influenced by Gab2, while PI3K regulates Ras function itself in BCR-ABL1 transformed cells. The Gab2/Grb2 adapter protein complex increases activity of the guanosine diphosphate/guanosine triphosphate (GDP/GTP) exchange factor Sos. This activation promotes accumulation of the active GTP-bound form of the small GTPase Ras. The Ras-Raf-mitogen-activated protein kinase (MAPK)/extracellular signaling-related kinase (ERK) cascade activates several effector kinases such as ERK1, ERK2, or the Jun N-terminal kinase (JNK), all of which contribute to gene transcription regulation in hematopoietic cells. Other pathways are also thought to be important in CML, for example progression to blast crisis is associated with inhibition of the phosphatase activity of the tumor suppressor PP2A as a consequence of the BCR-ABL1-induced expression of the PP2A inhibitor SET (57).

The second most frequent and biologically distinct *ABL1* fusion gene is *NUP214-ABL1* (58). Remarkably, this fusion is generated by circularization of the 500 kb genomic region from *ABL1* to *NUP214* and subsequent extrachromosomal (episomal) amplification. The fusion is cryptic cytogenetically and to date remains the only example of gene fusion by episome formation in malignancy. *NUP214-ABL1* is accompanied by increased *HOX* expression and deletion of *CDKN2A (p16)*, which are both common findings in patients with T-ALL. A study examining 279 adult patients with T-ALL found the *NUP214-ABL1* fusion gene in approximately 4% of all cases (59). However, the influence of *NUP214-ABL1* on survival remains unclear.

Other *ABL1* fusion genes have been described but are uncommon. *EML1-ABL1* was found in a single female patient with T-ALL and a cryptic t(9;14)(q34;q32) (60). The presence of this fusion was associated with a homozygous deletion of the tumor suppressor gene *CDKN2A* (*p16*) and expression of *TLX1* (*HOX11*). *EML1-ABL1* transfected BaF/3 cells become growth-factor independent. The *ETV6-ABL1* fusion gene is the product of a t(9;12)(q34;p13) and is found in occasional patients with acute leukemias or MPD (61). The oligomerization domain of ETV6 (TEL) was shown to be essential for leukemogenesis in a murine model (62). Recently, *ZMIZ1* and *RCSD1* were identified as *ABL1* partners in single cases of B-ALL (63, 64). An overview of currently known *ABL1* fusion partners in hematological disorders is presented in table 1.

TK fusion genes involving JAK2

Fusion genes involving *JAK2* occur at a much lower frequency than fusions involving *ABL1*. Intriguingly, the phenotype and clinical course of *JAK2* fusion genes more closely resemble leukemias associated with *ABL1* fusion genes (ie CML and ALL) rather than those with *JAK2* point mutations (classical MPDs). However, the clinical course of *JAK2* fusion

gene associated MPDs seems to be more aggressive than *BCR-ABL1*-positive CML as reflected by a shortened time to progression to blast phase. These observations clearly highlight the phenotypic importance of the mechanism leading to kinase activation, which appears to be even more important factor than the identity of the kinase.

Three different JAK2 fusion partners are currently known in hematological disorders. Similar to ABL1 and other TK fusions, they all retain the entire TK domain of JAK2, include an oligomerization domain provided by the partner genes, and show variability in their respective disease phenotypes. Currently, the most common fusion gene was initially reported in seven patients with a t(8;9)(p22;p24). In these rare cases, the 'human autoantigen pericentriolar material 1' (*PCM1)* gene, a gene from chromosome band 8p22 that encodes a large centrosomal protein, was translocated to the *JAK2* TK (65). Normal PCM1 is involved in recruitment of specific proteins to the centrosome during cell division. It is also found to fuse to another TK, the *RET* proto-oncogene, in papillary thyroid carcinoma (66). We have collected clinical and molecular data from 10 *PCM1-JAK2*-positive patients, and 7 additional patients have been reported in the literature (table 2). The median age at diagnosis of all 17 patients was 44 years (range, 12-74). Remarkably, all seven *PCM1-JAK2* cases in the initial report were male, and thus far only one of the 17 reported patients is female. A similar significant male bias is seen for patients with fusions involving the TKs PDGFRA and PDGFRB in MPDs (67). The reasons for this gender skewing remain unknown. *PCM1-JAK2*-positive patients present clinically as having a MPD (n=13), frequently with peripheral eosinophilia and/or myelofibrosis of the bone marrow. Alternatively, patients may present as *de novo* acute leukemia of lymphoid (n=3) or myeloid (n=1) origin. The involvement of *PCM1-JAK2* in both myeloid and lymphoid malignancy demonstrates that there is no clear lineage specificity to this fusion gene and is consistent with the idea that the *PCM1-JAK2* disease, like CML, originates from a hematopoietic stem cell.

The clinical course of *PCM1-JAK2*-positive patients is aggressive with rapid progression of chronic-phase disease (n=7, 54%) to secondary acute leukemia/blast phase of either myeloid (n=6) or lymphoid (n=1) origin, reminiscent of blast phase in *BCR-ABL1*-positive CML. Allogeneic stem-cell transplantation (SCT) was performed in 5 of 13 patients (38%) < 55 years either in first chronic phase (n=3) or in first remission after intensive chemotherapy of acute leukemia (n=2). Four of these patients are currently alive and progression-free at a median time of 33 months after transplant. One patient died 5 months due to graft failure. Twelve patients did not receive an allogeneic SCT, of whom five are alive and progression-free. Seven non-transplanted patients died at a median time of 12 months with only one death not being disease-related. Although case numbers are low, these data suggest that early allogeneic SCT should be considered for eligible *PCM1-JAK2*-positive patients with a suitable donor at the current time. In the future, JAK2 inhibitors may well become front line therapy for patients with *JAK2* fusions but their efficacy remains to be established.

Of interest, two *ABL1* fusion partners have been found to fuse with *JAK2. BCR-JAK2* was identified in a single patient with a t(9;22)(p24;q11.2), exhibiting a disease phenotype and clinical course similar to CML (68). *ETV6-JAK2* was identified in three patients with ALL and a balanced t(9;12)(p24;p13) or in a CML-like MPD with a more complex t(9;15;12)(p24;q15;p13) (61, 69). In a murine model, ETV6-JAK2 induced a fatal mixed myelo- and T-cell lymphoproliferative disorder (48). It is not known why some genes, such as *BCR* or *ETV6*, act as recurrent translocation partners in fusion genes and others, with obviously similar functional domains fail to translocate. In addition to the known importance of oligomerization domains (such as coiled-coil domains) for constitutive kinase activation, one could speculate that there might be additional unknown structural and functional properties of the partner gene contributing to functional fusion gene formation. Alternatively, they may be particularly prone to breakage and aberrant recombination.

ABL1 Mutations

In contrast to *JAK2, ABL1* point mutations have never been reported as pathognomonic events at the onset of disease, yet they have a central role in developing resistance to imatinib treatment in both CML and, even more frequently, in *BCR-ABL1*-positive ALL. *ABL1* mutations are believed to be present at diagnosis in a small population of *BCR-ABL1*-positive stem cells and give rise to a significant growth advantage when the unmutated clone is suppressed by imatinib. The relative frequency of secondary mutations in resistant CML patients ranges between 42 and 90% depending upon disease phase, but also upon the definition of resistance and the methodology used. The remaining cases acquire resistance through a variety of other mechanisms, not all of which are understood. *ABL1* point mutations variably reactivate the kinase activity of BCR-ABL1 leading to decreased sensitivity to imatinib by 3 to >100 fold. Typically, mutations block or impair imatinib binding whilst preserving the capacity to bind ATP.

The first mutation identified in resistant patients was the exchange of the amino acids threonine and isoleucine at position 315 (T315I) (70). Since then, more than 55 different point mutations encoding distinct single amino-acid substitutions have been identified in various regions of ABL1, including the P-loop, normally responsible for ATP-binding, the so-called T315 proximal region, the catalytic domain, and the activation loop (A-loop) connecting the N- and C-terminal lobes of the kinase domain (70-74). Although P-loop mutations were initially reported as being associated with a poor prognosis, (74) more recent data has suggested that the identity of individual mutations is more important that the region in which they arise (A. Hochhaus, personal communication). The clinically most relevant mutations include (listed in descending order for observed frequency): T315I, Y253F/H, ED255D/K/R/V, M351T, G250A/E and F359C/L/V (75, 76). These mutations exhibit a high degree of variation in sensitivity to increasing doses of imatinib or alternative TKIs, such as nilotinib or dasatinib. These observations have direct clinical relevance. The more common mutations, such as Y253, E255 and T315, confer high-level resistance to imatinib, whereas the less frequent mutations, such as M351T, F359V or L387M, mutations might be overcome by increasing imatinib dosage (77, 78). T315I, however is resistant to all three inhibitors and although candidate molecules have been described that can inhibit BCR-ABL^{T315I}, none are currently generally available for clinical use.

JAK2 Mutations

In 2005, five groups reported the identification of a single point mutation within JAK2 using distinct experimental approaches. This mutation is located at nucleotide 1849, where a guanine to thymidine substitution results in substitution of valine for phenylalanine at amino acid position 617 (JAK2V617F) (12-16). The JAK2V617F mutation is located within a highly conserved region of the autoregulatory JH2 pseudokinase domain, a region that is homologous to the true TK domain but lacks key catalytic residues. Impairment of JAK2 autoregulation caused by this mutation renders the JAK2 kinase constitutively active, possibly by becoming hypersensitive or even independent of incoming signals. The aberrant expression of JAK2V617F induces Epo-independent growth in human progenitor cells *in vitro* (79). In contrast to the BCR-ABL1 oncoprotein which can function in a receptor-independent manner, JAK2V617F modulated signaling is dependent on the presence of a Type I cytokine receptor even when the cognate ligand is not present (80). Expression of JAK2V617F by retroviral bone marrow transduction/transplantation in the hematopoietic system of lethally irradiated mice resulted in the development of a PV-like disease, accompanied by leukocytosis but normal platelet counts (12, 47, 81).

The JAK2V617F mutation is found in approximately 95% of patients with PV and 50% of patients with ET and PMF. It is also present in approximately 5-15% of patients with

atypical CML or MDS/MPD (82, 83) and in approximately 50% of patients with ‘refractory anemia with ring sideroblasts and thrombocytosis’ (RARS-T) (84-87). It is much less common in patients with other myeloid disorders such as MDS, CMML, CEL or AML (88, 89).

The relatively small proportion of patients who fulfil the WHO criteria for PV but are negative for JAK2V617F (<5%) were recently found to have base pair substitutions, deletions, insertions and duplications in *JAK2* exon 12 (table 3). The most frequently mutated amino acid sequences span from 542 to 544 (17, 90). In vitro studies demonstrated that *JAK2* exon 12 mutations activate multiple pathways that are associated with erythropoietin signaling. More importantly, one mutation studied in detail resulted in the development of a myeloproliferative phenotype in a mouse model. Patients with *JAK2* exon 12 mutations typically present with a myeloproliferative syndrome accompanied by isolated erythrocytosis with suppressed erythropoietin production (17, 90). In addition, base pair substitutions or deletions within the JH2 domain were identified not only in MPD but also in rare cases of acute leukemias and lymphoid disorders as summarized in table 3.

Until very recently, no other mutations in related JAK family TKs such as JAK1, JAK3 or TYK2 have been reported (14, 15, 82) though there has been evidence that mutations within JAK1 and TYK2 that are homologous of the JAK2V617F mutation render the kinase constitutively active and therefore may represent as yet undetected molecular defects in hematological disorders (91). However, Xiang et al. identified two novel JAK1 mutations (T478S, V623A) in two out of 94 patients with AML. These mutations did not transform Ba/F3 cells to growth factor independence but were shown to activate multiple downstream pathways *in vitro* (92).

In occasional patients, both *BCR-ABL1* and JAK2V617F have been detected (93-98). It is believed that at least in some of these cases the JAK2V617F mutation occurred before the acquisition of the Philadelphia (Ph)-chromosome but unfortunately material was not available in any case to determine if the two abnormalities are part of the same clone or whether these cases have acquired two distinct diseases. In addition, a single AML patient with a FLT3 internal tandem duplication (FLT3-ITD), which is a typical and important molecular aberration in this disease entity, was found in combination with the JAK2V617F mutation (99). More recently, a distinct subtype of systemic mastocytosis has been described that is both KITD816V and JAK2V617F-positive (100).

Somewhat surprisingly, the JAK2V617F mutation was reported to be present in healthy individuals in 1-10% of cases (101, 102), as has been described for *BCR-ABL1* (103, 104). One important difference however is that *BCR-ABL1* is only found at very low levels whereas JAK2V617F in normal individuals was detectable in some cases by sequence analysis (102), suggesting a mutant allele burden of at least 20%. Other groups, however, have not found JAK2V617F in normal individuals, possibly because of differences in sample size, detection methods and sensitivity (105, 106). The reported frequency of JAK2V617F in normal individuals is roughly 1000 times the incidence of MPDs and thus it is inconceivable that all positive cases will succumb to myeloproliferation, however Bellanne-Chantelot et al. reported JAK2V617F positivity of two healthy individuals who developed PV or ET after several years (107), supporting the hypothesis that additional acquired events must take place in order to develop a MPD following the JAK2V617F mutation (see below). JAK2V617F has also been detected in a small proportion of cases with lymphoma but it remains to be established if the mutation is really driving the disease in these cases (108).

Clinicopathological features of MPD with JAK2V617F

Soon after the initial discovery of the JAK2V617F mutation, it became clear that this molecular abnormality can be used as a clonal marker to detect the level in the stem cell hierarchy at which this mutation occurs. Initial results indicated that the JAK2V617F mutation may arise in a myeloid precursor cell because it could not be found in B- or T-lymphoid cells. The development of more sensitive examination methods by several independent groups, however, revealed that at least in some patients small fractions of clonal B-, T-lymphocytes and natural killer cells do actually carry the JAK2V617F mutation (12, 15, 109-112). Notably, all disease entities, PV, ET and PMF, showed lymphocyte involvement although this phenomenon was more common in PMF and PV. Therefore, it was concluded that the earliest affected cell must be a pluripotent non-committed stem cell with the potential for both myeloid and lymphoid differentiation. These results thus confirmed the long held belief that MPDs are stem cells disorders (113).

The mutated JAK2 kinase activates multiple signal transduction pathways contributing to transformation processes, including increased proliferation and/or inhibition of apoptosis. These signals include the phosphorylation of STAT proteins, notably STAT5 which in turn upregulates the anti-apoptotic BCL-X_L gene (114). Other pathways that have already been extensively characterized in leukemia models are the PI3K/mTOR and the MAPK/MAP pathway. Permanent expression of JAK2V617F in the erythroleukemic HEL cell line induces high levels of reactive oxygen species that are a well-studied factor in BCR-ABL1-positive CML that may contribute to genetic instability and disease progression (115-117). Furthermore, JAK2V617F reduces expression of the cell cycle inhibitor $p27^{KIP}$ and increases expression of cyclin D2, which, under normal conditions, promotes cell cycle transition (115). The suppressor of cytokine signaling 3 (SOCS3) is known to negatively regulate JAK2 mediated signaling by interfering with JAK2 and EpoR (118). However, constitutively activated JAK2V617F can give rise to hyperphosphorylation of SOCS3 and thereby escape its negative regulation effect, resulting in increased cellular proliferation (119). Therefore in this situation, SOCS3 acts more as an enhancer of JAK2 mediated signaling.

Leukemic transformation to blast phase/secondary AML occurs in a proportion of patients with PV, ET or PMF. The length of time from presentation to transformation/blast phase is dependent on the molecular abnormality with a hierarchy of *JAK2* fusions < *ABL1* fusions < *JAK2* point mutations. However, in contrast to blast phase associated with *ABL1* or *JAK2* fusion genes, which evolves through additional molecular aberrations in the fusion gene positive cell, blast cells obtained at leukemic transformation from JAK2V617F-positive disease are JAK2V617F-negative in approximately 50% of cases (120, 121). The reason for this is unclear, but may possibly reflect an underlying genetic stability that predisposes to different mutations. Alternatively, many consider that JAK2V617F is a secondary event (see below), at least in some cases, and JAK2V617F-negative transformation arises from the accumulation of additional genetic abnormalities in a partially transformed clonal pre-V617F cell. This has important clinical implications since blast phase of CML can be treated at least temporarily with imatinib or alternative TKIs, whereas a substantial proportion of patients in blast phase of JAK2V617F-positive MPDs are predicted to be unaffected by a JAK2 inhibitor. With the forthcoming availability of JAK2 inhibitors, a careful diagnostic work-up of blast cells has therefore to be undertaken in patients with leukemic transformation. Theoretically, effective treatment with selective JAK2 inhibitors might favor the outgrowth of JAK2V617F-negative leukemic blasts.

It remains largely unknown what mechanisms are responsible for the development of the distinct disease phenotypes associated with JAK2V617F in patients. It is apparent that the

individual's genetic background plays a pivotal role, as the use of different mice strains transduced with JAK2V617F resulted in the development of different disease phenotypes (81, 122). The majority of patients are heterozygous for the JAK2V617F mutation in their peripheral blood leukocytes. However many PV and PMF patients have myeloid and erythroid colonies that are homozygous for JAK2V617F. It has been demonstrated that the loss of heterozygosity at the *JAK2* locus is due to mitotic recombination, resulting in uniparental disomy for chromosome 9p (13). While JAK2V617F homozygous erythroid progenitors can be found in most patients with PV, their presence appears to be very rare in patients with ET (123). Expression levels of the mutated allele also seem to be relevant for the phenotype. The currently preferred theory indicates that low JAK2 kinase activity leads to ET while medium or high activity results in PV or PMF. Indeed, recent mouse models that were capable of inducing lower JAK2V617F expression in comparison to wild-type JAK2 developed a ET-like disease (124, 125). In contrast, conventional murine models that expressed JAK2V617F at higher levels resulted in phenotypes resembling PV with myelofibrosis and either absent or transient thrombocytosis (12, 81, 122). Alterations in the expression level of *JAK2* in patients can be achieved in different ways. Homozygous clones are believed to exhibit a higher proliferation rate than heterozygous clones, based on the finding that patients with PV and PMF have higher total cell numbers of mutated granulocytes than ET patients. It has been suggested that the presence of two mutated *JAK2* alleles might lead to increased downstream signaling. Some patients with PV and PMF display trisomy 9, hence the effect might be even more enhanced in this subgroup. In addition, epigenetic effects or variation in gene regulation may also account for differences in JAK2 kinase activity. Quite possibly, additional mutations might influence the cell's fate. However, the nature of such mutations is as yet unknown.

Is JAK2V617F sufficient to cause MPD?

In addition to the observation that transformed JAK2V617F-positive disease is often JAK2V617F negative, there are several other lines of evidence that suggest there may be other, as yet unknown genetic aberrations that precede the acquisition of JAK2V617F:

- **i.** In some MPD patients the percentage of JAK2V617F-positive cells is much smaller than the percentage of total clonal cell pool determined by X-inactivation assays (126) suggesting that an unknown factor drove clonality prior to JAK2V617F. However, the generality of this interpretation has been strongly challenged (121, 127).
- **ii.** The existence of JAK2V617F-negative erythropoietin-independent erythroid colonies (EECs) in PV also suggests that JAK2V617F actually plays a secondary role in disease pathogenesis (128), however other groups find that all EECs are JAK2V617F-positive, consistent with the mutation being a primary hallmark of PV (123).
- **iii.** In familial MPDs the JAK2V617F mutation is not inherited but always acquired and, importantly, is not found in all affected patients (129). Clearly some unknown genetic factor or factors is being inherited in these cases that predisposes to an MPD, but whether this factor plays any role in sporadic cases is not known. In breast cancer, for example, inactivating mutations of *BRCA1* or *BRCA2* are very strong predisposition factors, however mutations in these genes are very uncommon in sporadic tumors.
- **iv.** In some MPD cases it has been shown that all or nearly all granulocytes carry an acquired cytogenetic abnormality known to be associated with MPDs, such as 20q-, but the level of JAK2V617F is low (126) indicating that at least in these cases, JAK2V617F was secondary to the deletion. However it is far from clear whether

we can extrapolate from these rare cases to conclude that JAK2V617F is always or usually a secondary event.

There are two principal arguments against JAK2V617F generally being a secondary event. First, not a single patient has been identified who was JAK2V617F-negative at the time of MPD diagnosis, but became JAK2V617F later during the disease course of the disease (130). Second, JAK2V617F as a single agent rapidly gives rise to a PV-like disease in murine models (12, 47, 81) in a similar way to that which BCR-ABL1 gives rise to a CML-like disease. Although it is impossible to be absolutely certain, most people in the field consider that chronic phase CML is a one-hit disease, ie BCR-ABL1 is the sole abnormality. However the notion that BCR-ABL1 might also be second hit following clonal expansion of an unknown primary abnormality continues to smoulder in some quarters (131) and this issue will probably not be fully resolved until entire CML genomes are sequenced and compared to constitutional DNA.

Targeted therapy

Inhibition of ABL1

Targeting BCR-ABL1 with the selective TKI imatinib has indisputably marked a new era for the treatment of patients with newly diagnosed CML in chronic phase. Imatinib binds to and stabilizes the inactive form of BCR-ABL1. It inhibits BCR-ABL1 autophosphorylation as well as substrate phosphorylation, decreases proliferation, and induces apoptosis. Imatinib also possesses inhibitory properties against other members of the TK family, including PDGFRA, PDGFRB, ARG, and KIT and has been used successfully to treat MPDs with PDGFR fusion genes (132, 133).

Long-term studies of 454 chronic-phase patients resistant or intolerant to interferon showed complete cytogenetic responses (CCR) in 57% of patients after a median duration of treatment with imatinib 400 mg/d for 65 months. Progression-free and overall survival at 6-years follow-up were 65% and 76%, respectively. Patients who achieved at least a major cytogenetic response (MCR) at 12 months had an estimated 6-year survival of 90% (134). These response rates were accompanied by good tolerability and very few grade III/IV side effects (1-2%). At the 5-year follow up of the IRIS study, the estimated cumulative rate of CCR with imatinib was 87% with an overall survival of 89.6% (135). The risk of progression to accelerated or blast phase within five years was 7%, and the rate of CML-related deaths was only 5% with annual relapse decreasing to <1% in the fifth year of therapy (135). Ongoing national and international clinical trials are exploring ways to optimize therapeutic effects including using higher doses of imatinib of up to 800 mg, and combining imatinib with interferon or Ara-C.

Effective treatment surveillance by cytogenetic and molecular methods has become increasingly important for the optimal management of CML patients for the early identification of primary or secondary resistance. The levels of residual *BCR-ABL1* transcripts detected by quantitative reverse transcriptase-polymerase chain reaction (RQ-PCR) at certain time points are excellent predictors of achievement and durability of complete cytogenetic responses and favorable clinical course (136, 137). The kinetics of response in terms of speed and depth are highly variable and probably due to the heterogeneity of leukemia, as well as the variation in different pharmacokinetic and pharmacodynamic background of individual patients. Persistent *BCR-ABL1* positivity in the majority of patients even after years of treatment indicates that imatinib does not cure CML. Although some studies have reported complete molecular responses (CMR) which are even durable after stopping imatinib (138), *in vitro* studies have identified quiescent stem cells which are highly resistant to imatinib and probably survive even in patients with CMR

(139). It is therefore suggested that imatinib should be continued irrespective of achievement of a CMR. A significant problem of RQ-PCR results within and outside of clinical trials has been the lack of international standardization, however the proposal for a International Scale for *BCR-ABL1* measurement is being actively pursued and should improve comparability of results between laboratories (140).

Primary resistance in chronic phase is synonymous with failure of achieving CHR by 6 months, a MCR at 12 months, or CCR at 18 months. The proportion of patients who meet these criteria is estimated at 15-25% (141). Primary resistance is poorly understood, but there is increasing evidence that it is related to low plasma levels of imatinib resulting from impaired absorption or metabolism (142). Lack of compliance may also cause lower efficacy, but should not be seen as a real mechanism of resistance. Secondary resistance is synonymous to loss of response or progression of disease, and is estimated at 4% of patients per year, although this rate appears to be decreasing over time. Unsurprisingly, rates of secondary resistance are higher in advanced phases of CML. In addition to the reactivation of the dysregulated enzymatic activity of the BCR-ABL1 protein by secondary mutations as described above, overexpression of *BCR-ABL1*, activation of BCR-ABL1 independent pathways, higher activity of OCT-1 influx drug pump and overexpression of P-glycoprotein or heat-shock protein 70 have all been associated with imatinib resistance (136, 143, 144). Some of these mechanisms result in decreased imatinib plasma levels, recently associated with worse cytogenetic and molecular responses in chronic myeloid leukemia (145).

The second generation inhibitors nilotinib and dasatinib are more potent (lower IC_{50}) than imatinib towards the ABL1 kinase. These inhibitors demonstrate striking efficacy against almost all *ABL1* mutations, with exception of T315I (nilotinib and dasatinib) and Y253H (nilotinib) (146-148). Nilotinib is an anilio-pyrimidine derivative that is structurally related to imatinib and also requires the ABL1 protein to be in the inactive conformation for optimal binding. Like imatinib, nilotinib is also an inhibitor of other TKs such as PDGFRA, PDGFRB and KIT. However, nilotinib is most effective against ABL1, with an IC_{50} of 25 nM, making it approximately 20-30 fold more potent than imatinib. It has been suggested that the enhanced potency of nilotinib compared to imatinib is due to its higher affinity to the ABL1 kinase pocket. This is consistent with the nilotinib-ABL1 crystal structure, in which there is a better topographical fit of nilotinib to ABL1 due to increased contact with the binding surface. Nilotinib taken at a dose of 800 mg/d, led to a MCR in 48% and a CCR in 31% of 280 imatinib-resistant or -intolerant chronic phase CML patients with at least 6 months of follow-up (147). The same dose was used in 119 imatinib-resistant or -intolerant accelerated phase CML patients which led to a hematologic response in 47% and a MCR in 29% after a median treatment duration of 202 days (range, 2-611 days) (146). The overall survival rate after 12 months follow-up was 79%. Adverse events were generally mild to moderate in severity. The most common grade III/IV hematologic adverse events were thrombocytopenia and neutropenia. Further follow-up is however needed to assess long-term efficacy and side effects.

Originally designed as an immunosuppressant, the pyridol[2,3-d]pyrimidine kinase inhibitor dasatinib shows activity towards multiple additional kinases such as PDGFRA, PDGFRB, KIT, BTK, SRC family members plus a range of other kinase and non-kinase targets (149, 150). This compound is 300 times more active than imatinib and has the greatest potency against ABL1 kinase activity of all second-generation TKIs. It retains activity against all known imatinib-resistant BCR-ABL1 mutants *in vitro*, except T315I, which is completely resistant. The crystal structure of the dasatinib-ABL1 complex suggests that dasatinib binds to the ABL1 ATP-binding site in a position that is similar to imatinib. Unlike imatinib, dasatinib is able to bind to the active as well as inactive conformation of ABL1, which may partly explain the higher binding affinity to the ABL1 kinase. This binding profile, and also

that of nilotinib, probably explains why only a limited number of mutations are responsible for resistance to second generation TKIs in both model systems (151) and patients (152, 153). Dasatinib was shown to block G_1/S cell cycle transition and therefore to inhibit cell growth *in vitro*. The broader inhibition profile of dasatinib possibly explains the clinical side effects that are observed, including myelosuppression and fluid retention (e.g. pleural effusion). In 186 patients imatinib-resistant or intolerant CML patients treated with 140 mg dasatinib daily, a CHR was achieved in 90% of patients, while a MCR was seen in 52% (154). However grade III/IV neutropenias and thrombocytopenias were seen in approximately 50% of patients and grade III/IV pleural effusions in 6% of patients, (at a dose of 70 mg twice daily). Recently, it was shown that a daily dose schedule of 100 mg is equally effective but has many fewer side effects while toxicity-related treatment discontinuation could be reduced significantly (6 vs. 15%) (155). Currently, nilotinib and dasatinib are approved by the US FDA and EMEA for the treatment of chronic phase, accelerated phase and blast phase Ph-positive CML and Ph-positive ALL in adults following imatinib failure or intolerance.

In clinical practice, approximately 15-20% of patients in chronic phase who are treated with imatinib do not achieve CCR. In addition, some patients relapse following initial response. In such patients it is important to exclude compliance problems. Additionally, screening for point mutations of the ABL1 kinase domain should be undertaken, as should conventional cytogenetic analysis for the identification of clonal evolution. Depending on the results from these studies, further treatment options include increasing the dose of imatinib to 800 mg or switching to nilotinib or dasatinib. Currently, no data exist favouring one inhibitor or another and the decision should therefore also include an assessment of potential side effects in the individual patient. Other new dual TKIs are bosutinib (previously SKI-606) and INNO-406 (previously NS-187), which inhibit ABL1 and SRC kinases. Both inhibitors are significantly more active than imatinib against BCR-ABL1 and all known ABL1 mutants, with T315I being the exception. New experimental drugs must be considered under certain circumstances, such as the presence of a T315I mutation. MK-0457 (previously VX-680), an aurora kinase inhibitor, is active against BCR-ABL1 and all mutants including T315I (156). Also, allogeneic stem-cell transplantation should be reconsidered at this stage. In the future, a combination of two or more various kinase inhibitors may become an alternative treatment option for first or second line treatment of chronic-phase CML (157).

First and second-generation ABL1 kinase inhibitors are expected to achieve similar high rates of CHR and CCR in patients with myeloproliferative disorders and *ABL1* fusion genes other than *BCR-ABL1*. Because the majority of these cases have only been reported in individual patients, no data on the efficacy of imatinib are currently available. The situation may be different for the treatment of T-cell lymphomas/leukemias associated with *NUP214-ABL1* fusion genes. Response rates and risk of relapse may be similar to *BCR-ABL1*-positive ALL, in which imatinib, nilotinib, and dasatinib induce high response rates initially, but are unable to achieve durable responses with disease relapse occurring quite frequently within a short time (158).

Inhibition of JAK2

In contrast to *BCR-ABL1*-positive CML, no small-molecule JAK2 inhibitor is available for broad clinical use at the time of writing. However, numerous newly developed compounds, such as ITF2357, INCBO18424, TG101209, TG101348, lestaurtinib (formerly CEP701), MK-0457, AZD-1480, AZ-60, LS104, SB1518, and XL019 to name a few, have been extensively tested in pre-clinical studies on JAK2-dependent (HEL, Ba/F3^{JAK2V617F}) and JAK2-independent cell lines, in cultured human primary progenitor cells, and in murine models. Some of them (including INCBO18424, XL019, TG101348, lestaurtinib, and AT9283) are already being evaluated in early clinical phase-I/-II-studies in patients with

primary and secondary myelofibrosis. The precise mode of action is not fully understood for many of these compounds. It is possible that these inhibitors specifically interact with the ATP or substrate binding site or they could also exhibit unspecific behaviour as seen through treatment with histone deacetylase inhibitors (HDACi) or aurora kinase inhibitors. It was recently suggested to group these inhibitors to those which primarily target JAK2 as 'JAK2-selective' and those originally developed for other indications, such as myeloma, MDS, AML, but revealing significant 'off-target' inhibitory activity towards JAK2, as 'non-JAK2-selective' (159). Selective inhibitors would also target wild-type JAK2.

Because of the central role of JAK2 in hematopoiesis, JAK3 for the immune system, and the whole family of JAKs as pivotal downstream targets of the receptors of a large number of cytokines and growth factors, it may prove difficult to generate a specific inhibitor without undesirable long-term side effects. As an example, *JAK3*-deficient mice are viable but show a severe combined immunodeficiency (160). In contrast to *JAK1* and *JAK2, JAK3* is only expressed on hematopoietic cells. Therefore, it appears to be of particular importance to develop inhibitors that are specific to JAK2, and interfere with other JAK family members only minimally.

An example for a 'non-JAK2-selective' inhibitor is the novel HDACi compound ITF2357, which was originally reported as a potential promising agent for the treatment of AML and multiple myeloma. HDACi partly act through hyperacetylation of the NH2-terminal residue of the nucleosomal histones, which subsequently leads to transactivation of multiple transcription factors such as the cyclin kinase inhibitor $p21^{WAF1/CIP1}$, although the detailed mechanisms of their inhibitory potential towards cancer cells remains largely unclear. ITF2357 suppresses colony formation of JAK2V617F-positive cells, with an IC_{50} in the nanomolar concentrations, through specific downmodulation of the JAK2V617F protein. This induces inhibition of its downstream substrates causing a drastic decrease in phosphorylation of STAT3 and STAT5 while having to effect on wild-type JAK2 or STAT proteins in the K562 cell line. RQ-PCR revealed that JAK2V617F mRNA expression was not suppressed while *PRV-1* expression, a known target of JAK2, was rapidly downregulated (161).

TG101209 is an orally available inhibitor of JAK2, FLT3, and RET kinases. In addition to JAK2V617F, it also effectively inhibits growth of Ba/F3 cells and primary progenitor cells expressing the MPLW515L mutation with an IC_{50} of approximately 200 nM, much like JAK2V617F. Its therapeutic efficacy was also demonstrated in a nude mouse model (162). Similar growth inhibition patterns in HEL cells and primary cells from MPD patients characterized by inhibition of downstream targets such as STAT5 were observed with lestaurtinib and Gö6976, a indolcarbazole inhibitor of the calcium-dependent isozymes of protein kinase C (PKC) (163, 164).

Specificity in JAK2 inhibition is of particular relevance as impairment of endogenous JAK proteins could produce detrimental effects in vital pathways of the hematopoietic and immune system. Currently, the degree of selectivity of a JAK2 inhibitor for malignant versus normal hematopoiesis is unknown, but may be minimal. Several compounds have shown significant reduction in hematocrit, hemoglobin, reticulocytes, and splenomegaly in murine models of JAK2V617F-induced PV-like disease (165, 166). In general, a broader spectrum of drug inhibitor activity might be useful for simultaneous inhibition of several major oncogene-activated signaling pathways. However, this lack of specifity may also account for severe and yet unknown short- and long-term side effects. Unlike ABL1, which has no major role in myelopoiesis, JAK2 is required for erythropoiesis and probably terminal myelopoiesis (167). Some compounds, such as aurora kinase inhibitors, simultaneously inhibit several functionally relevant kinases and downstream targets within a

nanomolar range. Toxicological studies for JAK2 inhibitors are much anticipated, as no reliable data from human or animal studies currently exist.

Conclusions and future aspects

The various mutants of the non-receptor TKs *ABL1* and *JAK2* play a fundamental role in the pathogenesis of MPDs. The clinical phenotypes of fusion protein associated disorders are similar and comprise of CML, CML-like disorders, *de novo* acute leukemias of lymphoid phenotype, and blast phase/secondary acute leukemias of myeloid or lymphoid phenotype. Mutations of *JAK2* are a hallmark of PV and about 50-60% of cases with ET and PMF, whereas point mutations of *ABL1* have no role in the onset of disease, but are major contributors for the development of resistance to imatinib and alternative TKIs in CML.

The finding of identical molecular abnormalities in various disease phenotypes clearly highlights the impact of additional factors, which are important for our understanding of the pathogenesis and clinical course of these disorders. The individual genetic background, the affected cell compartment, and secondary unknown genetic events may be essential co-factors in addition to the mutation-specific activity of the TK, in a complex network leading to malignant cell proliferation.

Although the introduction of TKIs has brought substantial benefits for patients with *BCR-ABL1*-positive hematologic disorders, it currently appears unlikely that these malignancies can be cured, as TKIs fail to eradicate the quiescent leukemia-initiating cell. Additional strategies targeting the leukemic stem cell may prove beneficial in overcoming primary and secondary resistance. These approaches could incorporate simultaneous administration of several TKIs, inducing inhibition of other ABL1 domains essential for kinase function, such as substrate binding sites, targeting downstream signaling activated by the aberrant kinases or a combination of the various treatment options mentioned. For ABL1, many of the transduction pathways have already been characterized, whereas the detailed analysis of how JAK2 mutations confer their transformation properties has just begun. Novel JAK2 inhibitors are currently being evaluated *in vitro* and in phase-I/II clinical trials. Major primary obstacles include drug specificity for the various mutations without affecting wild-type JAK2 or related family members and toxicity effects in short and also long term usage. Additional problems might arise, as experienced with imatinib treatment of CML, with primary or secondary resistance and the failure to target the leukemic stem cell. Targeted therapy is very expensive and it will be important to develop markers to identify those patients who are most likely to benefit from treatment. Despite these concerns it is clear that targeted therapy has revolutionized the treatment of CML and there is considerable optimism that targeting JAK2 will bring similar benefits for patients with MPDs.

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Table 1

Fusion partner genes and phenotypes of *ABL1* in hematologic malignancies

Fusion Partner	Chromosome band	Phenotype	Reference
BCR	22q11	CML, ALL	(168)
ETV6	12p13	MPD, AL	(61)
EML1	14q32	T-ALL	(60)
NUP214	9q34	T-ALL	(58)
RCSD1	1q24	Pre-B-ALL	(64)
ZMIZ1	10q22.3	B-ALL	(63)

Table 2

Clinical phenotype of patients with the *PCM1-JAK2* fusion gene

Case	Male	MPD	Eosinophilia	Myelofibrosis	Acute Leukemia	T-NHL	Reference
#1	•	•		•	•		
#2	•	•	•	•			
#3	•	•	•	•	•		
#4	•				•		
#5	•	•	•	•			
#6	•	•					
#7	•	•			•		(65)
#8	•	•		•			
#9	•	•	•		•		(169)
#10	•	•	•		•		
#11					•		
#12	•	•	•				
#13	•	•	•				(170)
#14	•	•	•	•	•		(171)
#15	•					•	(172)
#16	•	•	•		•		unpublished
#17	•				•		unpublished
Overall	94%	82%	53%	35%	59%	6%	

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Table 3

Mutations within *JAK2* in hematological malignancies

Mutation	Exon (Domain)	Phenotype	Reference
V617F*	exon 14 (JH2)	PV, ET, PMF	(12-16, 82)
C618R	exon 14 (JH2)	PV	(173)
C616Y	exon 14 (JH2)	PV	(174)
L611S	exon 14 (JH2)	ALL	(175)
ΔIREED*	exon 14 (JH2)	B-ALL	(176)
K607N	exon 14 (JH2)	AML	(177)
D620E	exon 14 (JH2)	MPS, PV	(178, 179)
E627E	exon 14 (JH2)	MPD	(179)
T875N*	JH1	AMKL (cell line)	(180)
F537-K539delinsL*	exon 12	PV	(17)
H538QK539L*	exon 12	PV	(17)
K539L*	exon 12	PV	(17)
N542-E543del*	exon 12	PV	(17)
I540–E543delinsMK-	exon 12	PV	(181)
R541–E543delinsK-	exon 12	PV	(181, 182)
V536-I546dup11	exon 12	PV	(90)
F537-I546dup10+F547L	exon 12	PV	(90)
H538-K539delinsL	exon 12	PV	(182)

*Abnormalities that have been shown to have transforming activity in model systems