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SPOTLIGHT REVIEW

Chronic myeloproliferative diseases with and without the Ph chromosome: some unresolved issues

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Ph-positive chronic myeloid leukemia (CML) and Ph-negative chronic myeloproliferative diseases (MPDs), characterized in many cases by the presence of the *JAK2*^{V617F} mutation, have many features in common and yet also show fundamental differences. In this review, we pose five discrete and related questions relevant to both categories of hematological malignancy, namely: What are the mechanisms that underlie disease progression from a relatively benign or chronic phase? By what therapeutic methods might one target residual leukemia stem cells in CML? Is *JAK2*^{V617F} the original molecular event in MPD? What epigenetic events must have a role in dictating disease phenotype in MPDs? And finally, Will the benefits conferred by current or future *JAK2*^{V617F} inhibitors equal or even surpass the clinical success that has resulted from the use of tyrosine kinase inhibitors in CML? These and others questions must be addressed and in some cases should be answered in the foreseeable future.
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Introduction

Progress in understanding the biology of chronic myeloproliferative diseases (MPDs) continues apace. Although in recent years there was a tendency to regard Ph-positive chronic myeloid leukemia (CML) as an entity quite distinct from Ph-negative myeloproliferative diseases, the categorization proposed originally by Dameshek in 1951, whereby all the so-called myeloproliferative diseases (MPDs) form part of a spectrum with perhaps more similarities than differences, increasingly proves to be 'correct'. In this paper, we have considered very recent developments in five closely related areas, namely the molecular mechanisms that might underlie the progression of CML from the chronic phase (CP) to blastic transformation, some of the approaches that might be useful to target residual CML stem cells in treated patients on the assumption that they may have the capacity to regenerate the complete clinical picture of CML, the question whether JAK2^{V617F} is the initial molecular event in MPDs, the issue of what sort of molecular or epigenetic lesions might define the clinical phenotype in JAK2^{V617F}-positive disorders and finally, newer approaches to targeting *JAK2* in clinical practice. In each case, there seem to be lessons that could apply equally to both Ph-positive and Ph-negative diseases.

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What causes CML to progress?

Treatment of early CP CML with imatinib (IM) significantly reduces its rate of progression,[1,2] but transformation is still a major therapeutic challenge as in the majority of those patients who do progress to blast crisis, the response to tyrosine kinase inhibitor (TKI) therapy is not durable.[3,4] This is often attributed to the heterogeneous nature of the advanced phase of CML,[5] in which various chromosomal and molecular abnormalities are usually present, some of which may have a role in maintaining the transformed state, such as by the inactivation of tumor suppressor genes.[6,7] In the TKI era, the molecular changes observed comprise deletions, insertions or point mutations involving various genes, including BCR-ABL1.[6,7] Such genetic lesions including DNA copy number aberrations appear to occur predominantly in blast crisis, more commonly in lymphoid than in myeloid crisis; in contrast, genetic lesions have been identified more rarely in patients with CP or accelerated-phase CML.[6] In most patients with lymphoid blast crisis, the most frequent mutations occur at CDKN2A/B (50–67%), whereas in myeloid blast crisis, mutations in the p53 locus occur in ~30% of patients, but not a single genetic lesion has yet been identified at high frequency.[5,6,8] However, it is worth noting that in experimental mice, specific blast crisis-associated chromosomal translocations (e.g., NUP98-HOXA9) can transform a BCR-ABL1-induced myeloproliferative-like phenotype into a blast crisis-like disease.[9]

Despite the nonrandom nature of some of the cytogenetic changes observed in advanced-phase CML, it is unlikely that any specific secondary chromosomal abnormality can directly cause disease progression. The precise molecular events predisposing to blastic transformation are also unknown, although activation of Wnt and Hedgehog signaling and inhibition of the protein phosphatase 2A (PP2A) may all make major contributions.[10–17]

The molecular changes mentioned above could be secondary as evidence is now being accumulated to support the concept that the greatly increased *BCR-ABL1* kinase activity in $CD34^+$ granulocyte-macrophage progenitors (GMPs) could be the primary determinant of disease progression,[5–7] as a series of epigenetic changes that determine the phenotype of blast crisis $CD34^+/CD38^+/CD45RA^+$ GMP seems to depend on increased *BCR-ABL1* activity.[5,18] The differentiation arrest of GMPs depends on the ability of high *BCR-ABL1* activity to activate mitogen-activated protein kinase (extracellular signal-regulated kinase 1/2) that, in turn, enhances the translational inhibitory effect of the RNA-binding protein hnRNP E2 on C/EBPα, which is the major regulator of myeloid maturation.[19] Similarly, the self-renewal ability of GMP results from BCR-ABL1-independent[20] and BCR-ABL1-dependent[10,21] signals leading to the inhibition of GSK 3β (glycogen synthase kinase 3β) and consequently to the activation of β-catenin, and mutations that generate a mis-spliced and thereby inactive GSK3β mRNA have been detected in ~50% of blast crisis patients.[12] Furthermore, *BCR-ABL1* dramatically perturbs the CML transcriptome resulting in altered expression of genes, of which some (e.g., PRAME, MZF1, EVI-1, WT1 and JUN-B) might also have a role in disease progression.[6,11,22,23]

The post-transcriptional, translational and post-translational effects of high levels of *BCR-ABL1* may also be important. They may result in the activation of factors with reported mitogenic and anti-apoptotic activity (e.g., *MYC, JAK2, LYN, STAT5, BMI-1, PI-3K/AKT* and *BCL-2*-related proteins) as well as in the inhibition of major key regulators of cellular processes, such as p53 and PP2A.[5–7] Indeed, the suppression of PP2A is essential for the induction and maintenance of *BCR-ABL1*-generated oncogenic signals and also allows *BCR-ABL1* to be expressed at high levels.[24] These postulated *BCR-ABL1* dose-dependent mechanisms of altered gene regulation, clearly not limited to those cited above, may explain blastic transformation in CML patients who do not seem to have any chromosomal or molecular abnormalities, and may also explain the relative sensitivity, at least initially, of blast crisis cells to TKIs. Interestingly, the *BCR-ABL1* oncoprotein increases the incidence of point mutations and chromosomal aberrations by simultaneously inducing pathways that lead to the accumulation of free radicals causing oxidative damage and altering the efficiency and faithfulness of the DNA repair machinery.[25] In this regard, the acquisition of resistance to TKI has also been attributed to the *BCR-ABL1*-induced genomic instability and cells presenting with high *BCR-ABL1* activity show decreased sensitivity to IM and frequently develop TKI-resistant mutant subclones.[26,27] As resistance to TKI that was independent of *BCR-ABL1* but dependent on the *SRC* family member *LYN* kinase has also been reported,[28] TKI resistance might also be a factor that facilitates disease progression.

Furthermore, it is quite possible that the genetic instability of CML blast crisis depends both on an increased propensity of CML CP progenitors to undergo genetic changes and on the probability that one of the mutations induced by *BCR-ABL1* or by some other mechanism functions as an 'amplifier' of a genetically unstable phenotype. Indeed, there is also a cohort of CML patients (~15%) presenting with deletions of the derivative chromosome 9, whose leukemia may be more prone to genomic instability *ab initio*;[29] such patients progressed to blast crisis much more rapidly than CML patients lacking the der9 deletion, although this may not be the case in the 'imatinib era'. The same considerations may apply to the recently described *GATA-2* L359V mutation and to the loss of chromosome Y observed in CML patients who were undergoing blastic transformation.[30,31]

To date, strong evidence supports the idea that the dosage of BCR-ABL1 kinase activity has a pivotal role in many CML patients undergoing progression, and that in some cases secondary genetic or chromosomal abnormalities can facilitate transformation, whereas in other cases they just influence the aggressiveness of the already transformed CML blast crisis progenitor cell clone. Indeed, ~30% (pre-IM era)[32,33] and ~50% (post-IM era)[34] of blast crisis patients do not show chromosomal abnormalities and presumably only a fraction of these patients have molecular inactivation of the p53 gene, yet their overall survival is only marginally better than that of patients with chromosomal abnormalities. Thus, the crucial answered question is, 'What controls *BCR-ABL1* expression and activity during progression?' A possible scenario might include a *BCR-ABL1* autoregulatory loop that amplifies signals, which positively influence *BCR-ABL1* gene transcription and enhance its protein stability by preventing its proteasome-dependent degradation. Conversely, we cannot exclude the possibility that there could be a single genetic lesion, still unidentified, that occurs in CP CML with high frequency and predisposes to blastic transformation.

Targeting residual leukemia stem cells

Various lines of evidence support the conclusion that leukemia stem cells (LSCs) are rarely, if ever, eradicated by treatment with TKIs. Although TKIs induce rapid hematological and cytogenetic responses in the majority of CP CML patients,[1,2] relapse of the disease is generally observed when TKI treatment is interrupted. A recent study conducted on 14 CML patients treated with IM for a minimum of 4 years showed that *BCR-ABL1* levels were maintained in primitive hematopoietic stem cell (HSC) ($CD34^+$, $CD38^-$ fraction) despite continuous IM treatment.[35] Mathematical models have been used to analyze the *in vivo* kinetics of disease response to TKIs in CML patients, providing a quantitative understanding of the CML cell dynamics with respect to mutation, selection and genetic instability. These models support the idea that TKIs are potent inhibitors of the production of differentiated CML cells, but do not completely eradicate CML.[36]

All CP CML patients present with a subpopulation of stem cells that are quiescent and are characterized by a primitive phenotype ($CD34^+$, $CD38^-$, HLA DR^-, $CD45RA^-$, $CD71^-$), which are not targeted by TKIs even at high concentrations (reviewed in the study by Pellicano and Holyoake[37]). The presence of such TKI-resistant LSCs could explain the residual disease in optimally responding patients and the relapse observed in most patients who discontinue TKIs. The mechanism(s) that allow these cells to be insensitive to TKIs remains unclear. Although in a few cases the presence of mutations in the kinase domain of LSCs may explain their drug resistance, such mutations cannot be detected after exposure to drug *in vitro*, and when relapse occurs after TKI discontinuation the relapse is the usual *BCR-ABL1* fusion gene without mutations,[38] implying that the relapse in these cases was not due to LSCs with IM-resistant kinase domain (KD) mutations.

Several new strategies to target CML stem cells are now under investigation. The principal target of TKIs is proliferating cells; hence, one possible approach to eradicating primitive quiescent CML cells would be to stimulate cell cycle entry before or during treatment with TKIs. *In vitro* pulsing with growth factors, such as G-CSF (granulocyte-colony stimulating factor), promotes cell cycle reentry before and after treatment with IM and significantly improves stem cell targeting in comparison with IM

alone.[39] Although this particular study provided solid data for a randomized, multicenter, pilot study to investigate the effect of G-CSF treatment together with intermittent IM, clinical results did not show a benefit for either pulsed IM or pulsed IM in combination with G-CSF.[40]

Another approach is based on the notion that induction of autophagy may have a pro-survival effect. If so, the use of autophagy inhibitors, such as chloroquine, might enhance the therapeutic effects of TKIs in targeting CML.[41] We have shown that IM treatment *in vitro* induces autophagy in primary CML cells, including stem cells. Furthermore, by suppressing autophagy, either by knocking down essential autophagy genes or with pharmacological inhibitors, it is possible to promote cell death induced by IM.[42]

In addition to targeting BCR-ABL1, other areas of research include investigating drugs that target molecular pathways commonly defective in cancer. One class of anticancer drugs that has received much attention is the farnesyl transferase inhibitors, designed to target the activation of oncogenes, including *RAS. BCR-ABL1* activates the RAS signaling pathway, which promotes enhanced proliferation and malignant transformation. A farnesyl transferase inhibitor developed by Bristol-Myers Squibb, Wallingford, CT, USA (BMS-214662) significantly reduces quiescent CML stem cell numbers by the induction of apoptosis.[43] It synergizes with TKIs to overcome the anti-proliferative effect exerted by these agents on CML stem and progenitor cells.[43] Although BMS-214662 exerts a strong farnesyl transferase inhibitor activity *in vitro*, its overall mechanism of action in killing the LSC fraction is still unclear.

Another drug with a strong potential for targeting quiescent CML stem cells is the sphingosine analog FTY720, a powerful activator of the tumor suppressor PP2A.[17,44] At the last ASH meeting, Perrotti's group reported that *SET*-dependent suppression of PP2A activity is a common feature of Ph-positive progenitors and IM/dasatinib-insensitive $CD34^+/CD38^-$ *BCR-ABL1*-positive stem cells but not of the equivalent cell fractions obtained from healthy individuals.[45] By clonogenic, colony-forming cell (CFC)/replating, long term colony-forming cell (LTC-IC) and carboxyfluorescein succinimidyl ester (CFSE)-mediated cell division-tracking assays, they showed that FTY720 (2.5 μM) suppresses survival and self-renewal and triggers apoptosis of *BCR-ABL1*-positive stem/progenitor cells isolated from the bone marrow of CML blast crisis patients and/or from SCL-tTA-BCR-ABL transgenic animals. It must be noted, FTY720 did not exert any significant effect on the $CFSE^{MAX}$ quiescent stem cell fraction obtained from healthy individuals. Perrotti's group also showed that BCR-ABL1-independent PP2A-regulated signals control the survival and self-renewal of CML stem cells through a mechanism that may involve the activation of β-catenin that may be a PP2A target essential for the self-renewal of the CML blast crisis GMPs.[20]

Finally, Pandolfi and colleagues have suggested a new approach to cancer therapy that targets non-proliferating cancer stem cells. They showed that when hematopoietic stem cells derived from mice that lacked the tumor suppressor promyelocytic (PML) leukemia protein are first transduced with BCR-ABL1 and then transplanted into irradiated recipients, thus inducing leukemia, the quiescent LSCs rapidly enter cell cycle which leads to LSC exhaustion.[45] As the cells start to proliferate, they become more sensitive to chemotherapeutic drugs. In their study, such mice (i.e., PML null LSC) treated with cytarabine achieved complete remission of leukemia. Interestingly, in the same study, CML patients with low levels of PML expression had a good clinical outcome. Therefore, another possible approach to the elimination of residual LSC could be to target PML.

Is JAK2-V617F the initiating lesion in Ph-negative MPDs?

When the mutated $JAK2^{V617F}$ is expressed in bone marrow cells by retroviral transduction[46–50] or in transgenic mice.[51–55] MPD phenotypes ranging from thrombocytosis to polycythemia and in some cases myelofibrosis can be observed. These results have been interpreted as evidence that MPD is initiated by $JAK2^{V617F}$ as a single-step process. However, several observations suggest that in patients with MPD, the situation is more complex and that other mutations may precede the acquisition of $JAK2^{V617F}$ [54–56] The first surprise was that in many patients with essential thrombocythemia (ET), but also polycythemia vera (PV) and primary myelofibrosis (PMF), the $JAK2^{V617F}$ mutation was present in only a small proportion of cells and could be detected only by a sensitive real-time PCR assay.[57,58] In patients with a mutant $JAK2^{V617F}$ allele burden of $<25\%$, the granulocytes that did not carry $JAK2^{V617F}$ were frequently clonal, as determined by the X-chromosome inactivation pattern in females or by the presence of deletions on chromosome 20q.[55] These data suggested that in some MPD patients, somatic mutations in genes other than *JAK2* precede the acquisition of the $JAK2^{V617F}$ mutation. Similar conclusions were reached by comparing the relationship between granulocyte clonality and $JAK2^{V617F}$ allelic ratio.[54] A correlation between clonality and the $JAK2^{V617F}$ allelic ratio was shown for PV but not for ET or PMF.[54] The finding of endogenous erythroid colonies with wild-type *JAK2* in MPD patients with $JAK2^{V617F}$ [56,59] and studies on familial MPD that showed an inherited predisposition to acquiring somatic mutations in $JAK2^{V617F}$[60] further strengthened the concept that genetic alterations may precede $JAK2^{V617F}$. An analysis of single colonies obtained from patients positive for $JAK2^{V617F}$ and del(20q) showed that del(20q) could occur before or after the acquisition of $JAK2^{V617F}$ [61]. Therefore, del(20q) is unlikely to be a predisposing event for acquiring $JAK2^{V617F}$

The presence of two different mutations in the same patient can represent sequential clonal evolution, that is, both mutations are acquired sequentially in the same cell. Alternatively, the two mutations may represent two independent clones (bi-clonal disease). Evidence for bi-clonal disease was obtained by analysis of single colonies in a patient with $JAK2^{V617F}$ (exon 14) and a *JAK2* exon 12 mutation.[59] Furthermore, MPD patients with mutations in the thrombopoietin receptor, *MPL*, frequently also carry $JAK2^{V617F}$. In a larger series, $JAK2^{V617F}$ and *MPL* mutations represented bi-clonal disease in all six cases studied.[62] The occurrence of two rare events, such as $JAK2^{V617F}$ and *JAK2* exon 12 mutation or $JAK2^{V617F}$ and *MPL*-W515K/L, in two different progenitors from the same patient further supports the idea that patients with MPD carry a predisposition to acquiring rare somatic mutations. Such predisposing mutations could be acquired, affecting blood cells only, or could be inherited through the germ line. Evidence for the latter model was obtained by studying X-chromosomal inactivation in single colonies from female patients with bi-clonal disease.[62] Interestingly, several groups recently reported that $JAK2^{V617F}$ mutations preferentially occur on one of the two chromosomes 9 that carry a haplotype defined by a series of single-nucleotide polymorphisms within the *JAK2* gene.[63–65] Although this association is statistically highly significant, the increased risk of acquiring $JAK2^{V617F}$ in carriers of the 46/1 haplotype (also known as the CCGG haplotype) is only moderately increased (relative risk = 2.6). It remains to be determined whether the $JAK2^{V617F}$ mutation preferentially arises on chromosome 9 with the 46/1 haplotype or whether the $JAK2^{V617F}$ mutation on the 46/1 haplotype has a selective advantage and more frequently initiates MPD.

Somatic mutations in *TET2*, a gene of as yet unknown function, have been detected in ~14% of MPD patients and in ~30% of patients with myelodysplastic syndrome, as reported very recently.[66–70] Frame shifts, stop codons or substitutions of conserved amino acids were detected either as heterozygous or homozygous mutations. Mutations in *TET2* were also observed in familial cases of MPD, but the mutations in these family members were acquired and not inherited through the germ line. TET2 gene mutations could precede the acquisition of *JAK2*^{V617F}, but the opposite order of events cannot be excluded. The detailed report on this very interesting new gene must be awaited, and a number of questions regarding the role of these mutations in MPD will have to be addressed in the future.

Novel mechanisms in MPDs

The seminal discovery of the *JAK2*^{V617F} mutation early in 2005 followed by the description of *JAK2* exon 12 and *MPL* mutations has facilitated the diagnosis and has also improved the knowledge of the pathogenesis of MPDs, particularly that of polycythemia vera. However, it was soon recognized that this mutation, although integral to the myeloproliferative process in murine models, may not be the sole and even sufficient molecular event. In the last couple of years, experimental support has been provided to each of the main four different theories currently advocated, alone or in combination, to explain the puzzle of 'one mutation-different diseases', that are: (1) a different stem cell as the target of the mutation; (2) variable levels of *JAK2* kinase activity as a reflection of the relative proportion of mutated and wild-type protein in cells; (3) the unique genetic background of the host; and (4) a pre-*JAK2* molecular event. These points were critically reviewed by James[71] at the 2008 ASH meeting, and the possible role of pre-*JAK2*^{V617F} events, including novel mutations in *TET2*, a putative tumor suppressor gene located at 4q24, discovered recently in Vainchencker's laboratory,[66] is mentioned above. Therefore, although the search for other genetic defects in patients either positive or negative for *JAK2* and *MPL* mutations is actively pursued by adopting high-throughput genomic approaches, there has been a growing interest in the last couple of years in the study of 'post-genomic' abnormalities that might contribute to or cause the phenotypic variability of the disorders, including regulation of genes at the epigenetic level or mediated by microRNAs, and post-translational protein modification.

Epigenetic abnormalities in cancer cells point to cell-heritable defects that affect gene expression and occur as a result of two main mechanisms, namely DNA methylation and modifications (acetylation or methylation) of histones.[72] A large body of information exists regarding both hypermethylation at specific gene loci, particularly of tumor suppressor genes, and global DNA hypomethylation in cancer cells from solid tumors or hematological neoplasia. However, studies on epigenetics in MPDs are still scanty, but there is increasing evidence indicating that some genes, which are supposedly involved in MPD pathogenesis, can be abnormally regulated at the epigenetic level. For example, reduced levels of *SOCS3*, a member of the family of suppressors of cytokine signaling which function as negative regulators of the *JAK2* signaling pathway, have been reported in cells obtained from MPD patients, particularly those with PMF, and have been ascribed to promoter gene hypermethylation.[73,74] Another example is the SDF1 receptor CXCR4, which is abnormally downregulated in the CD34$^+$ hematopoietic progenitor cells that constitutively circulate in PMF patients. The reduced transcriptional activity of CXCR4 is caused by hypermethylation at specific CpG islands of the promoter, that reverted to normal state after short-term incubation with the demethylating agent 5-aza-deoxycitidine; furthermore, a significant reduction in the proportion of *in vitro*-generated *JAK2*^{V617F} mutated cells was observed after long-term incubation of CD34$^+$ cells with a combination of 5-azacitidine and an histone deacetylase (HDAC) inhibitor.[75] These treatments also resulted in the correction of the abnormal *in vitro* migratory characteristics of CD34$^+$ cells [76] and in their seeding in the bone marrow of NOD/SCID mice.[77] However, a global methylome profile of MPD cells is not yet available, and the significance of these observations still needs to be confirmed. In this regard, it is of interest that the constitutively increased activation of the JAK–STAT pathway can promote epigenetic silencing of genes important for cellular transformation, according to findings that over-activation of the *Hopscotch* gene, a JAK homolog gene, caused a global disruption of heterochromatic gene silencing and tumor formation in *Drosophila melanogaster*.[78] Thus far, the question whether targeting epigenetic mechanisms in MPDs is a useful therapeutic strategy has only been addressed in a few small clinical trials. 5-Azacitidine was used in patients with refractory/relapsed PMF and post-polycythemic/post-thrombocythemic MF in two Phase II trials differing in drug scheduling,[79,80] whereas the preliminary results of a Phase II multicenter study with decitabine in PMF have also been reported;[81] however, only minimal clinical responses were recorded. On the other hand, a novel HDAC inhibitor, ITF2357, showed promising clinical activity in a Phase II trial, particularly in PV and ET patients, accompanied by evidence of a progressive decline of cells harboring the V617F allele.[82] *In vitro*, the drug significantly reduced proliferation of *JAK2*^{V617F} mutated cells, including endogenous erythroid colony formation, through the post-transcriptional downregulation of JAK2.[83]

Orchestrating gene expression in normal adult cells and during development is one role of microRNAs (miRNAs), which is a large family of small non-coding RNAs. However, miRNAs can also function either as oncogenes or oncosuppressors[84] in human cancer, including acute or chronic leukemias. There are data indicating that miRNAs may be abnormally regulated in MPD cells, usually by a general downregulation as reported in other cancer cells. Conversely, some miRNAs were found to be overexpressed and this apparently correlated with the *JAK2*^{V617F} mutation;[85,86] in particular, there are preliminary data suggesting that miRNA-16 can be involved in the abnormal expansion of erythroid compartment in PV.[87] However, we need more information about miRNA gene targets and the mechanisms underlying their differential expression in MPDs.

Additional complexity can originate from abnormal post-translational protein modification, as recently discovered by Green and colleagues[88] who studied deamidation of the anti-apoptotic protein Bcl-x_L, which is known to be upregulated in both CML and PV. Recent data indicate that a BH3 mimetic peptide induces apoptosis in *JAK2*^{V617F} high-allele burden PV erythroblasts, preventing their proliferation and inhibiting the generation of endogenous erythroid colonies.[89] In normal cells, Bcl-x_L deamidation in response to etoposide or radiation-induced DNA damage is a mechanism used for deleting mutated cells through a DNA damage-induced apoptotic pathway. It was found that induced Bcl-x_L deamidation is prevented in *JAK2*^{V617F} or *BCR-ABL* mutated cells; the fact that incubation of PV or CML myeloid cells with *JAK2* inhibitors or IM, respectively, restored the Bcl-x_L deamination pathway, would support a causal link between the defective deamination response and the aberrant tyrosine kinase activity. It is tempting to speculate that the accumulation of clonal cells harboring

SPOTLIGHT

damaged DNA due to the inadequacy of Bcl-x_L deamination might facilitate the stepwise progression of PV or CML to acute leukemia. Additional support for the role of defective modifications of key proteins in MPD cells is derived from the observation that *SOCS3* is unable to exert its negative regulation of JAK/STAT signaling because the turnover of the protein is reduced, unlike that of SOCS1; in fact, exogenous *SOCS3* was actually found to promote, rather than to reduce, the proliferation of murine cell lines expressing *JAK2*^{V617F} possibly as a consequence of V617F-induced protein hyperphosphorylation.[90]

Do JAK2 inhibitors represent the future for MPD therapy?

Ph-negative MPDs: a need for better therapy

The therapy of the Ph chromosome-negative MPDs, namely ET, PV and PMF, is at an exciting crossroad where on the one hand there is an explosive increase in our understanding of their pathogenetic mechanisms and on the other hand there is rapid evolution of targeted therapies designed to block these mechanisms. Until now, the treatment of the Ph-negative MPDs has been by far most effective in the earliest phases of disease. Specifically, for PV and ET agents such as anagrelide and hydroxyurea have clearly reduced the risk of both thrombotic and hemorrhagic events. However, such drugs have not been very valuable in the later phases of disease, especially for those patients with late-phase primary myelofibrosis or myelofibrosis arising after earlier ET or PV (post ET–PV–MF).[91] Indeed, current therapies have been unable to prevent progression to either these phases or to acute leukemia.[91] Allogeneic stem cell transplantation can cure patients with advanced MPD disorders, but is still associated with an appreciable risk of short- and long-term morbidity and mortality; moreover, the increasing average age of patients with MPDs means that only a minority are really good candidates for transplantation procedures.

The discovery of several key MPD-associated mutations has broadened the therapeutic horizons for these disorders significantly.[92] Starting with the discovery of the *JAK2*^{V617F} mutation in exon 14 of *JAK2*, there are presently 10 mutations described in *JAK2* exon 12. In addition five mutations thus far have been identified in the thrombopoietin receptor *MPL*. All of these mutations seem to feed into a final common pathway of cellular activation through the PI3 kinase pathway, the STAT pathway and the mitogen-activated protein kinase pathway.[92]

Various therapeutic strategies are being developed for attempting to block the proliferative stimulus associated with these MPD-associated mutations. Current testing of the therapeutic inhibition of these inhibitors can be classified into three groups, specifically pre-clinical (based on the *in vitro* activity against *JAK2*^{V617F}-containing cells), those with ongoing testing in murine models and those undergoing testing in clinical trials. Although there is a pipeline of between 10 and 20 agents with reported *in vitro* or murine model activity, we will focus on those agents in which clinical activity has already been reported in the public forum. The clinical results *JAK2* inhibition can be divided into three categories of agents. The first comprises novel small molecules designed and tested for specificity and selectivity against *JAK2* (INCB 018424, XL019, TG101348). The second comprises agents that inhibit various kinases, including *JAK2* (ITF2357, CEP-701). The third comprises agents that have previously shown clinical activity in Ph-MPDs in which their impact on *JAK2* (and on *JAK2*^{V617F}) allele burden is being measured, such as pegylated interferon α-2.

JAK2 inhibitors for myelofibrosis

The most mature clinical experience for a JAK2 inhibitor is for INCB018424 (Incyte Co, Wilmington, DE, USA) (selective against JAK1 and JAK2) with the largest PMF trial in history (>120 patients). This agent leads to significant reduction in splenomegaly and dramatic improvement in constitutional symptoms.[93] Although a well-tolerated drug, the suppression of the JAK–STAT pathway (including normal hematopoiesis which signals through this pathway) can lead to treatment-related thrombocytopenia and anemia.[92] Additional drugs being tested are early in their results (TG101348—selective JAK2 inhibitor (TarGen, San Francisco, CA, USA),[94] XL019—selective *JAK2* inhibitor (Exelexis, San Francisco, CA, USA),[95] CEP-701 (TKI of *JAK2* and *FLT3*) (Cephalon, Frazer, PA, USA),[96] ITF2357 (histone deacetylase inhibitor) (AQ18Italfarmaco, Italy)[97] but preliminary results also report improvements in splenomegaly and symptoms in MF patients (Table 1). No *JAK2* inhibitor has yet reported a significant ability to improve cytopenias, fibrosis or histological changes associated with MF. There is not yet a clear difference in terms of efficacy between selective and non-selective *JAK2* inhibitors. Pathogenetically, what separates MF from PV and ET is not yet clear, but is probably not solely the currently identified *JAK2* or MPL mutations. This latter fact could explain why a *JAK2* inhibitor could lead to only a partial response in MF patients, akin to the more limited ability of IM mesylate to achieve response in accelerated or blast-phase CML.

JAK2 inhibitors for PV and ET

PV (with 99% of patients having a mutation somewhere in their *JAK2*) could well be the most straightforward target of *JAK2* inhibition, and preliminary results of trials with XL019[98], CEP-701 (Cephalon),[99] and ITF2347[97] show activity in decreasing erythrocytosis. However, these trials are too early in their accrual to allow any useful conclusions as to their efficacy. Interestingly, in PV, the agent that has shown an ability to lead to significant reductions in *JAK2*^{V617F} allele burden in 30–40% of patients (including complete molecular remissions) is pegylated interferon-α-2.[100]

Table 1 Currently reported efficacy for JAK2 inhibitors from clinical trials in patients with myelofibrosis

	Anemia	*Splenomegaly*	*Constitutional symptoms*	*Pruritus*	*⇓JAK2 burden*	*Myeloproliferation*	*Reference*
INCB018424	<10%	X	X	X	10%	X	Verstovsek *et al.*[93]
CEP-701	<10%	X	X	—	Not reported	X	Verstovsek *et al.*[96]
XL019	—	X	X	X	10-20%	X	Shah *et al.*[95]
TG101348	—	X	X	X	Not reported	X	Pardanani *et al.*[94]
ITF2357	—	X	X	X	8-12%	X	Rambaldi *et al.*[97]

Future impact of JAK2 inhibitors

The future of MPDs seems bright because of the level of excitement and resulting scientific effort directed toward studying them. The *JAK2* inhibitors bring great excitement to the field of Ph-negative MPDs because of their targeted approach. However, although this pipeline of agents that inhibit *JAK2* is strong, will any of the agents discussed or in development achieve remissions or alter the course of MF (or even PV/ET)? Presently, *JAK2* inhibitors have provided a valuable and incremental benefit over existing options particularly for symptoms and quality of life, but have no impact yet on anemia or more advanced disease features. Preliminary evidence is encouraging, although it too early to know the true impact that these agents will have on the proliferative aspect of the disorders or the risk of disease progression, and nothing is known about whether these agents will decrease the thrombotic and hemorrhagic risks, both major problems in PV and ET. In a changing landscape for Ph-negative MPDs these new agents, or subsequent generations, may have a significant role whether this role will entail the use by themselves or in combination with existing therapies.

Conflict of interest

The authors declare no conflict of interest.

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Participants

The following individuals were present at the meeting in Sonoma, California: Ralph Arlinghaus, Houston, USA, Tiziano Barbui, Bergamo, Italy, Olivier Bernard, Paris, France, Raj Chopra, Astra-Zeneca, Liverpool, UK, Connie Eaves, Vancouver, Canada, Oliver Hantschel, Vienna, Austria, Ron Hoffman, New York, USA, Robert Gale, Los Angeles, USA, Alan Gewirtz, Philadelphia, USA, John Goldman, London, UK, Tony Green, Cambridge UK, Rudiger Hehlmann, Mannheim, Germany, Tessa Holyoake, Glasgow, UK, Catriona Jamieson, San Diego USA, Xiaoyan Jiang, Vancouver, Canada, Robert Kralovics, Vienna, Austria, Ross Levine, New York, USA, Paul Manley, Novartis, Ruben Mesa, Scottsdale, USA, Tariq Mughal, London, UK, Alfonso Quintas-Cardama, Houston USA, Heike Pahl, Freiburg, Germany, Danilo Perrotti, Columbus, USA, Giuseppe Saglio, Torino, Italy, Radek Skoda, Basel, Switzerland, Richard Silver, New York, USA, Tomasz Skorski, Philadelphia, USA, Simona Soverini, Bologna, Italy, Ted Szatrowski, Bristol-Myers Squibb, USA, Alessandro Vannucchi, Florence Italy, Rick van Etten, Boston, USA, Richard Woodman, Novartis.

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