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Current pre-clinical and clinical advances in the *BCR-ABL1*-positive and -negative chronic myeloproliferative neoplasms

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Though it has been remarkable to have witnessed the major advances in the understanding of the molecular pathogenesis of the chronic myeloproliferative neoplasms (MPN) over the past three decades, many challenges remain. The advances began with the identification of the *BCR-ABL1* gene in chronic myeloid leukemia (CML) in 1985, leading to the introduction of ABL1 tyrosine kinase inhibitors (TKIs), and the *JAK2*^{V617F} mutation in polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) in 2005, leading to the JAK inhibitors.¹⁻³ CML is now arguably the most successfully treated human malignancy. Despite these remarkable achievements, the quest for cure, functionally defined as treatment-free remission after discontinuation of TKI therapy, remains difficult.⁴ In the *BCR-ABL1* negative MPNs, a similar degree of success has not been achieved, perhaps because of the rather surprising clonal complexity of these disorders and the increasing molecular evidence of the need for *JAK2*^{V617F} mutation to cooperate with other genetic aberrations in the initiation and progression of the disease. This clonal complexity needs to be elucidated further in order to recognize clinically relevant candidate therapeutic targets. Herein, we review some of the topical challenges and successes in the biology and therapy of the MPNs that were discussed at the 7th post-American Society of Hematology CML-MPN workshop, which took place in Atlanta on December 11-12, 2012, and up-dated prior to this publication.

1. Unraveling the impact of epigenetics in the classical MPNs

It has been speculated that some of the heterogeneity in CML might be attributable to differences in patients at the epigenetic level. Few studies have addressed the DNA methylome in CML. Dunwell *et al.* identified two genes (*TFAP2* and *EBF2*) that showed increased methylation at the time of blast crisis, while hypermethylation of the autophagy-related gene *ATG16L2* was associated with poorer response to TKI therapy.⁵ Jelinek *et al.* studied the incidence of abnormally methylated promoters in 10 selected genes, the frequency of which was shown to increase during advanced phase disease and following resistance to imatinib. They showed that abnormal methylation of the SRC

suppressor gene *PDLIM4* was associated with shorter overall survival independently of disease phase or imatinib responsiveness.⁶ A recent study showed aberrant hypermethylation of *CEBPA* gene promoter and a significant association of this hypermethylation with the CML stage.⁷

Bazeos *et al.* collected CD34⁺ cells from newly diagnosed patients with CML in the chronic phase of disease and compared their DNA methylation profiles with analogous cells collected from the same individuals at the time of achieving at least complete cytogenetic response (CCyR) as well as from healthy controls (A Bazeos *et al.*, personal communication, 2012). Unsupervised hierarchical clustering revealed a series of differentially methylated probes, which were associated with untreated CML and represented a CML signature. Profiles obtained from patients who had achieved CCyR following treatment with tyrosine kinase inhibitors showed similar DNA methylation patterns to those of healthy persons with some residual CML signature marks. The investigators correlated their finding with clinical parameters but were unable to demonstrate major differences in methylation patterns between newly diagnosed individuals and concluded that the CML methylation signature is remarkably homogenous. It is possible that the CML phenotype requires acquisition of a *BCR-ABL1* fusion gene in association with a series of specific epigenetic changes.

There is also interest in assessing the relationship between microRNA (miR) expression and CML, particularly in patients who are resistant to TKI treatment. Patients responding to imatinib have demonstrated an increased expression of some miR (-150 and -146a) and decreased expression of other miR (-142, -199b, -145).⁸ Earlier studies also demonstrated an association between a downregulation of miR-203, possibly due to CpG methylation (or simply genomic instability), and the *ABL1* upregulation in newly diagnosed patients.

Studies on the genetic architecture of MPN have discovered an unexpected high level of complexity. Most mutant genes in MPN fall in three functional classes: JAK/STAT signaling (mutations in *JAK2*, *MPL*, *CBL*, *LNK*, and rare mutations in *SOCS*), epigenetic gene regulation (*TET2*, *EZH2*, *ASXL1*, *DNMT3A*, *IDH1*) and spliceosome factors (*SRSF2*).⁹ Patients with MPN who transform to acute myeloid

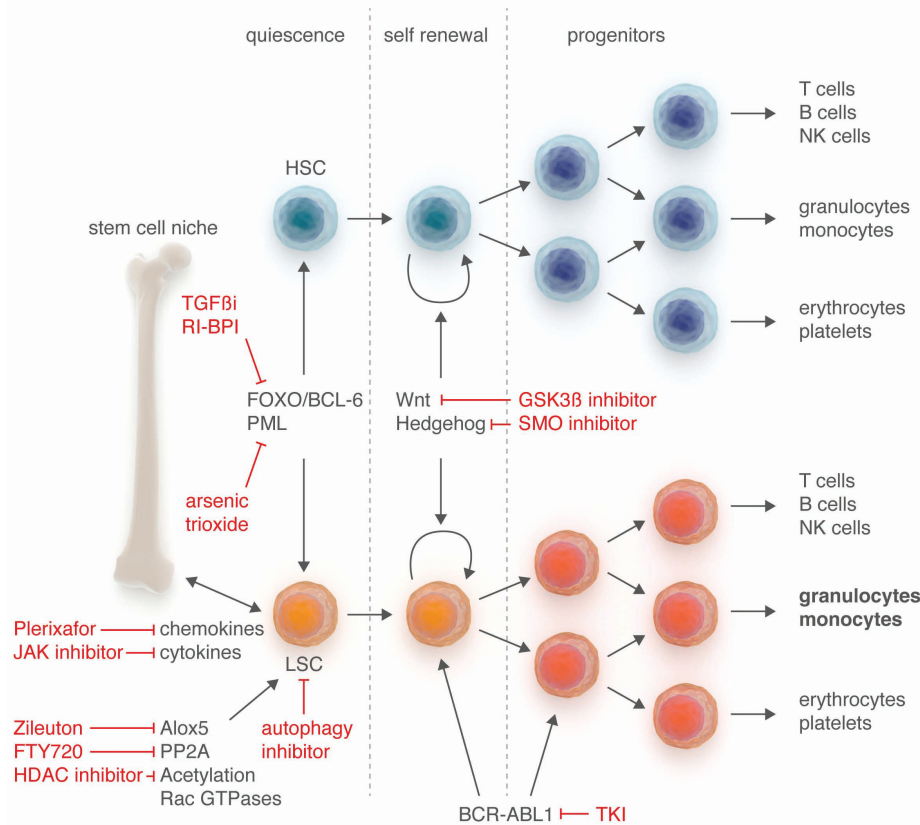


Figure 1. Agents that could be added to TKIs in CML. Pathways and targets implicated in the maintenance of CML stem cells are illustrated, with potential therapeutic interventions in red boxes. Courtesy of Professor Tessa Holyoake, Glasgow, UK.

leukemia (AML) often acquire additional mutations, such as *TP53*, *MDM2*, *IKZF1* and several others.¹⁰

Mutations in genes encoding epigenetic regulators are uncommon in MPN and unlike the *JAK2* and *MPL* mutations, which are demonstrable in most, but not all, patients with MPN and correlate with the subtype phenotype, no clear patterns have emerged. Aberrations in *ASXL1*, *EZH2*, *SRSF2* and *IDH1/2* appear to have a predictive impact on the overall and leukemia-free survival, suggesting the notion of the MPN epigenome to be clinically relevant, and the greatest impact appears to be due to *ASXL1* mutation.¹¹ Other candidate epigenetic modulators include the *CXCR4* promoter acquiring methylation defects, such as those resulting in constitutive migration of CD34⁺ cells in PMF. Recent studies using genome-wide DNA methylation arrays demonstrate diverse methylation patterns in the various subtypes of MPN, which could motivate efforts to target the MPN epigenome. Nischal *et al.* showed patients with PV and ET to be characterized by aberrant promoter hypermethylation, in contrast to those with PMF, who exhibit aberrations in both hypo- and hypermethylation.¹² Pahl and colleagues have also demonstrated the notion of epigenetic silencing of *AML-2* in diverse MPNs, and overexpression of *JMJD1C* in PV, to contribute in the overexpression of *NF-E2* and transformation to AML in murine models.^{13,14}

Furthermore, this seems to be associated with genes, in particular *ASXL1*, mostly involving inflammatory pathways in PMF, but not PV or ET. It was of interest to note that, in this study, the *JAK2*^{V617F} mutation did not appear to play a significant role in the MPN-epigenome. There is also much interest stimulated by recent observations of somatic mutations in calreticulin (*CALR*) gene in *JAK2* and *MPL* unmutat-

ed PMF and ET, but not PV.^{15,16} Much work is now needed to establish the precise molecular and clinical relevance of *CALR* mutations. Additionally, *in vitro* studies revealed successful inhibition of the aberrant methylation in PMF-derived cell lines by decitabine, supporting candidacy of this drug for clinical trials. In contrast, Perez *et al.* demonstrated the DNA methylation pattern to be similar in chronic phase PV, ET and PMF, and differentially methylated genes were mostly enriched in the NF-κB pathway.¹⁷ They also noted that in MPN patients who transform to AML, the number of differentially methylated regions increased significantly and the aberrant genes were involved in the 'interferon pathway' or were represented by genes already reported in *de novo* AML. Collectively, these efforts suggest underlying epigenome differences between the MPN subtypes with potential clinical impact, and provide motivation for the use of hypomethylating and other novel agents targeting the epigenome in the MPNs.

2. CML stem cells and efforts to target them

The currently licensed TKIs are remarkably effective in killing proliferating mature CML cells, but it is now clear that CML stem cells are probably not dependent on BCR-ABL1 for survival, and so alternative strategies targeting pathways that regulate the survival and maintenance of CML stem cells are required. Candidate pathways that appear to be activated by BCR-ABL1 include the JAK-STAT, mTOR, PI3K/AKT and autophagy signaling pathways (Figure 1). Additional areas of interest include the newly described Abelson helper integration site 1 (AHI-1), an adapter protein which interacts with BCR-ABL1 and *JAK2* to mediate the response of CML cells to imatinib, and the

mechanisms by which CML stem cells interact with their microenvironment.¹⁸ Several groups have recently demonstrated the successful targeting of both *BCR-ABL-1* and *JAK2* in the CML stem cells in *in vitro* models of CML stem cells procured from imatinib-resistant patients.¹⁹ The notion of combining ABL-1 TKIs and JAK inhibitors, however, remains uncertain at this time.²⁰

Other candidate pharmacological interventions of interest include innovative methods in targeting the bone marrow microenvironment which can function as a protective factor against TKI-induced apoptosis of CML stem cells and several diverse strategies, including the combination of TKIs with other candidate inhibitors which target diverse signaling pathways, including WNT/ β -catenin, Hedgehog, TGF- β /FoxO3a/BCL6 and JAK2/PP2A. When considering bone marrow microenvironment, it is particularly important to consider the marrow niche, a physico-chemical space that not only protects the stem cells, but also appears to play a major role in the trafficking and retention of these cells via the chemokine receptor CXCR4 and its ligand CXCL12.^{21,22}

3. What is the clinical impact of the JAK inhibitors in patients with myeloproliferative neoplasms?

The treatment algorithm for most patients with MF, but not the other subtypes of MPN, has changed dramatically following the recent introduction of the JAK inhibitors into clinical practice.^{23,24} Long-term follow-up data of the 2 randomized phase III COMFORT trials with the JAK1/JAK2 inhibitor ruxolitinib confirm the efficacy of the drug, irrespective of the presence or absence of the *JAK2*^{V617F} mutation, in ameliorating symptoms, reducing splenomegaly and according a modest survival benefit. The overall safety of the drug has also been confirmed, with hematologic toxicity, primarily anemia and thrombocytopenia, representing the most common side-effects. Anemia is an on-target side effect of JAK2 inhibitors, with the exception of momelotinib (formerly CYT387; Gilead), which well may have a unique hematologic profile.²⁵ About half of the transfusion-dependent subjects with MF enrolled in a phase I/II trial of CYT387, with a follow up at 2.5 years, obtained durable transfusion independence, and a general decrement of the percentage of all subjects requiring RBC transfusions as compared to baseline was also observed. The reasons for this intriguing unique characteristic of CYT387 compared with the other JAK inhibitors are unclear. All of the currently reported clinical data on the JAK2 inhibitors in patients with MF show considerable efficacy in reducing splenomegaly and ameliorating the constitutional symptom burden. It is generally considered that the abnormally increased inflammatory cytokines are a target of JAK2 inhibitors, but the precise details of their clinical impact still have to be clarified.

Additionally, a topical challenge is to address the impact of the JAK inhibitors on the natural history of MF, and indeed other MPNs. Currently there is no firm consensus on the impact of these drugs on the allelic burden of *JAK2*^{V617F} or on the extent of bone marrow fibrosis. Vannucchi *et al.* reported that patients receiving ruxolitinib had an overall approximately 8% reduction in *JAK2*^{V617F} allele burden at week 72 (range -51% to 15%); however, a subset of patients (22%) presented allele burden reduction greater than 20% that correlated with achieving the primary end point of more than 35% reduction in spleen size, suggesting that some patients

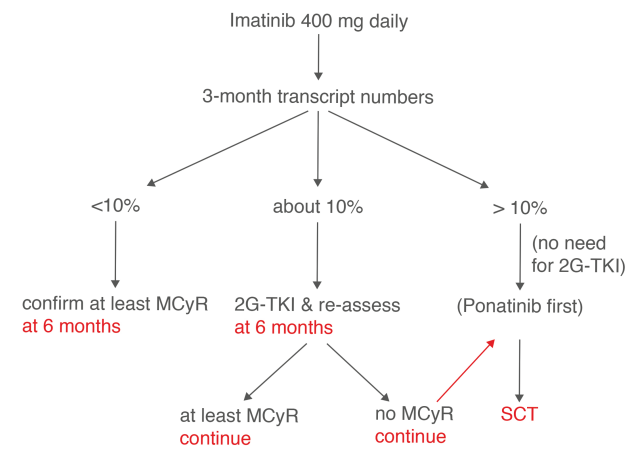


Figure 2. Potential management schema for a patient with CML-CP who has a possible transplant donor. Management is based in part on the assessment of *BCR-ABL1* transcript levels at three months after initiation of imatinib therapy. A value below 10% can be taken as an indication to continue imatinib; a value substantially above 10% may indicate TKI resistance and SCT should then be considered. A value in the region of 10% may suggest the need try a 2nd generation TKI before opting for SCT. Courtesy of Professors Tariq Mughal and John Goldman.

might be more sensitive to JAK2 inhibitor treatment.²⁶ Kvasnicka *et al.* presented preliminary evidence suggesting a reduction of bone marrow fibrosis in a cohort from an earlier phase II study with ruxolitinib.²⁷

The combination of JAK inhibitors with other molecules targeting different pathways is also being addressed at the pre-clinical and clinical levels. Candidate drugs include histone deacetylases, inhibitors of HSP90, the PI3K/mTOR pathway and of hedgehog signaling. Their activity as monotherapy and in combination with JAK inhibitors is supported by analysis both *in vitro* and in mouse models, and phase I/II trials combining ruxolitinib with panobinostat (an HDAC inhibitor), BKM120 (a PI3K inhibitor), LDE225 (a hedgehog inhibitor), or decitabine (a DNA methyltransferase inhibitor) are currently underway. Efforts are also being directed to addressing issues related to acquired resistance to JAK inhibitors.²⁸

4. What is the prognostic impact of the *BCR-ABL1* transcripts levels at three months?

Much interest has focused on the value of *BCR-ABL1* transcript levels measured at three or six months following the initiation of TKIs as first-line and subsequent therapies.²⁹ Patients with *BCR-ABL1* transcript levels of less than 10% at three months appear to have significantly increased rates of CCyR and CMR, and longer progression-free and overall survival compared to patients with higher transcript levels (>10% on the International Scale (IS)). Several groups have now independently validated this “Early Molecular Response (EMR)” milestone.³⁰ The 2013 European LeukemiaNet (ELN) CML guidelines, which are not dissimilar to the 2013 NCCN guidelines, recommend molecular response to be assessed at three, six, and 12 months. *BCR-ABL1* transcript levels of less than 10% at three months, less than 1% at six months, and less than 0.1% from 12 months onward represent an optimal response.³¹

5. Can TKI therapy be discontinued safely in patients with CML who have achieved a complete molecular response?

It is generally accepted that TKI therapy should be continued indefinitely in patients with CML who are responding optimally and tolerating the treatment well. The unresolved challenge, therefore, is really how long to continue treatment, which is of additional interest given that most patients have persistent CML stem cells, and the ability of TKIs to 'cure' remains uncertain. For the patient who has achieved a CCyR, stopping the drug usually leads to recurrence of *BCR-ABL1*-positivity and eventually, if left untreated, cytogenetic relapse in a substantial proportion. The French STIM (Stop Imatinib), the European Stop Kinase Inhibitor (EURO-SKI) trials and the Australian CML8 study (TWISTER) are probably the best efforts currently addressing this challenge.^{32,33} In the 36-month update of the STIM study, it was observed that the overall probability of remaining in complete molecular response (CMR, equivalent to a >4-log reduction in *BCR-ABL1* transcript levels) at 36 months after have stopped taking imatinib was 39% (95%CI: 29-48). Most of the patients who experienced molecular relapse did so within six months of discontinuing imatinib, and importantly, responded promptly to the reintroduction of imatinib, suggesting that discontinuation did not result in an acquired resistance. The efforts so far have identified patients with a low Sokal risk score, male sex, and longer duration of imatinib treatment as potential prognostic factors for the maintenance of CMR after discontinuing imatinib. Currently, it is probably best to discontinue the TKI therapy only within the framework of a clinical trial.

Conclusions

For patients with CML in chronic phase, there are now five TKIs in the clinics: imatinib, nilotinib, dasatinib, bosutinib and ponatinib. The first three are licensed for first-line use, in addition to other drugs such as interferon and omecetaxine. Figure 2 depicts a potential current treatment algorithm for the newly diagnosed patient in chronic CML who has failed first-line imatinib therapy (JM Goldman and TI Mughal, personal communication, 2013). Both of the 2nd-generation TKIs, dasatinib and nilotinib have, so far, fared considerably better than imatinib in randomized studies of first-line treatment for patients in terms of achieving a higher rate of CCyR and MMR, but not in terms of progression-free survival or overall survival.³⁴ One of the current challenges, therefore, is to establish whether the next generation TKIs might provide an overall survival which is significantly better than 85% at ten years. This will be a daunting task of recruiting large numbers of patients into large randomized prospective trials that would require lengthy follow up at a considerable cost. Moreover, unlike many of the current TKI trials, it will be useful if the future trials could have homogeneous definitions of the different end points and events.

There is persuasive evidence that imatinib 600 mg daily is tolerated in more than 80% of CML patients and results in superior cytogenetic and molecular responses at 12 and 24 months compared to the conventional 400 mg daily dose.³⁵ Regardless of the dose of imatinib, the current safety analysis of imatinib is quite impressive, with very few potentially serious long-term effects, including those concerning quality-of-life issues. Side-effects include nausea, headache, rashes, infraorbital edema, bone pains, and,

sometimes, more generalized fluid retention. Efforts are ongoing to develop suitable strategies which should facilitate the safe discontinuation of therapy for patients who achieve a CMR.³⁶ This issue is clearly important from a cost perspective also.³⁷ Each of the newer agents may be associated with the development of adverse events, some of which can be serious and include peripheral arterial occlusion and arterial thrombosis.

In sharp contrast with the deep molecular response and the durable long-term remission of CML with TKI, the impact of JAK2 inhibitors in patients with MF is, so far, limited to the control of splenomegaly and symptomatic improvement, with scarce current evidence in support of deeper effects on disease pathophysiology. Yet, these agents have a profound, unforeseen impact on the quality-of-life of patients with MF, and the overall improvement also provides survival benefit. These agents are relatively safe, and the hematologic toxicity reflects the essential non-redundant role of JAK2 signaling in normal hematopoiesis. Nonetheless, the follow-up period is relatively short and there are some concerns with regards to optimal dose. Acquired clinical resistance to JAK2 inhibitor therapy is being observed, possibly due to novel mutations, and might represent a challenge for the future therapies. Combining JAK inhibitors with other targeted drugs, including immunomodulators, epigenetic agents and telomerase inhibitors, are now being pursued, in tandem with efforts to unravel the underlying molecular complexities.³⁸

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Acknowledgments: the participants of the 7th post-ASH Workshop on CML and MPN were: Chairs: J.M Goldman, T.I Mughal & A. Tefferi; Plenary speaker: W. Vainchenker; Faculty: O. Abdel-Wahab, T. Barbui, G. Barosi, O. Bernard, J. Crispino, G. Finazzi, C. Gambacorti-Passerini, J. Gotlib, P. Guglielmelli, R. Hehlmann, O. Hantschel, C. Jamieson, J.-J. Kiladjan, G. Martinelli, M. Mauro, D. Perrotti, L. Pieri, G. Saglio, T. Skorski, S. Soverini, J. Thiele, R.A. Van Eten, A.M. Vannucchi. This workshop was made possible by unrestricted educational grants from Incyte Corporation, Bristol-Myers Squibb and YM Bioscience. Special thanks to Dr Alpa Parmar and the Alpine Oncology Foundation team for their help with the workshop logistics.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

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