

The Biology Behind

Understanding the Molecular Basis of Imatinib Mesylate Therapy in Chronic Myelogenous Leukemia and the Related Mechanisms of Resistance¹

Commentary re: A. N. Mohamed *et al.*, The Effect of Imatinib Mesylate on Patients with Philadelphia Chromosome-positive Chronic Myeloid Leukemia with Secondary Chromosomal Aberrations. *Clin. Cancer Res.*, 9: 1333–1337, 2003.

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Introduction

CML³ is a myeloproliferative disorder with an incidence of approximately 1–1.5 cases/100,000 population/year, a slight male predominance, and a peak between 50 and 60 years of age (1). This condition arises from a transformed pluripotent hematopoietic precursor associated with t(9;22)(q34;q11.2) that gives rise to the Ph' chromosome. At the molecular level, this cytogenetic aberration results in the fusion of the *ABL* gene at chromosome band 9q34 with the *BCR* gene at chromosome band 22q11.2. The resultant *BCR/ABL* fusion gene encodes a chimeric protein necessary and sufficient to confer the leukemic phenotype. In a minority of the cases, despite absence of the Ph' chromosome, molecular methodologies (*i.e.*, fluorescence *in situ* hybridization, Southern, or RT-PCR) can still detect *BCR/ABL* as the product of complex cytogenetic aberrations involving other chromosomes in addition to 9q34 and 22q11.2, or cryptic genomic rearrangements of the *BCR* and *ABL* genes. Taken all together, >95% of the CML cases are associated with *BCR/ABL* expression that, therefore, represents the hallmark of this condition.

CML is a bi- or triphasic disease (2). Patients usually present in a chronic proliferative phase characterized by splenomegaly and accumulation of neutrophils in their various stages

of maturation. Basophils and blasts are normally <20% and 10%, respectively, in CP CML. Evolution to the BP (or blast crisis) is defined by an increase ($\geq 20\%$) in myeloid or lymphoid blasts in blood, BM, or extramedullary locations. In many cases, the transformation is characterized by a passage through an AP, in which the failure of a patient to respond to therapy is accompanied by an increase in percentage of blasts (10–19%); a $\geq 20\%$ increase in basophils, uncontrolled thrombocytosis, or thrombocytopenia; and acquisition of new cytogenetic abnormalities such as trisomy 8, isochromosome 17q, or a second Ph chromosome and/or genetic inactivation of the *p53* gene.

The ultimate goal of treatment for CML is prevention of blast crisis, because, once this occurs, the prognosis is dismal. Although a rapid reduction of the white cell count can be achieved with chemotherapy (*i.e.*, busulfan or hydroxyurea) in CP CML, these drugs usually fail to prevent disease progression. IFN- α was the first agent proven capable of modifying the biological history of CML by prolonging survival in patients who achieved CHR and MCR (<35% of Ph' + cells; Ref. 3). A second breakthrough in the treatment of CML occurred when 60–80% of patients undergoing alloBMT in CP were reported to be disease free at 5 years (4–6). However, both IFN- α and alloBMT have considerable treatment-induced toxicity that attenuated the initial enthusiasm for these results, and novel, less toxic therapeutic strategies are being explored. In 2001, Druker *et al.* (7) reported the first Phase I study with imatinib mesylate (STI571, Gleevec), a specific inhibitor of *BCR/ABL* oncogenic activity. In this study, the authors demonstrated that high rates of CHR and MCR were achieved with relatively few side effects in patients with CML refractory or intolerant to IFN- α , opening new avenues for molecularly targeted therapies in this disease. Subsequently, encouraging results were also obtained for CML patients in BP (8).

Is *BCR/ABL* the Driving Force for Leukemogenesis in CML?

The transforming activity of *BCR/ABL* has long been demonstrated using *in vitro* and *in vivo* models. In initial studies, transfection of the *BCR/ABL* gene fusion resulted in malignant transformation of normal fibroblasts, and induced independent survival and proliferation in growth factor-dependent cell lines. Expression of *BCR/ABL* was also shown to be necessary and sufficient to induce leukemogenesis in animal models (reviewed in Ref. 9). Expression of *BCR/ABL* in mice was achieved by either introduction (“knock-in”) of the fusion gene in the mouse genome or by infecting murine stem cells with *BCR/ABL*-containing retroviral vectors. In knock-in transgenic mice with

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³ The abbreviations used are: CML, chronic myelogenous leukemia; Ph', Philadelphia; ABL, Abelson; RT-PCR, reverse transcription-PCR; BM, bone marrow; CHR, complete hematologic remission; MCR, major cytogenetic response; alloBMT, allogeneic bone marrow transplantation; AGP, $\alpha 1$ glycoprotein; CP, chronic phase; AP, accelerated phase; BP, blastic phase.

conditional *BCR/ABL* expression, a low penetrance phenotype of acute B- or T-cell leukemia was reported. In contrast, in mice sublethally irradiated and transplanted with syngeneic retrovirally transfected *BCR/ABL*+ stem cells, a condition mimicking human myeloproliferative disorders with neutrophil increase, BM expansion, hepatosplenomegaly, extramedullary hematopoiesis, and pulmonary leukostasis was observed.

How Does BCR/ABL Transform Cells?

The fusion partner *ABL* is a member of the nonreceptor tyrosine kinase family (10). This gene encodes a protein containing a tyrosine kinase activity domain (SH1) in addition to two other regulatory domains (SH2 and SH3) that mediate protein-protein interaction and modulate activation of signal transduction. A nuclear localization domain is also present, supporting a shuttling activity of the *ABL* protein between cytoplasm and nucleus. Genetic disruption of *ABL* in mice results in lymphopenia, runting, and perinatal mortality. The other fusion partner, *BCR*, is a protein with multiple functional domains involved in oligomerization, SH-2 binding, serine/threonine kinase activity, and activation of members of the Rho small GTP-ase family of proteins (10). Structural analysis of this gene suggests a role as a mediator of signaling transduction. With targeted disruption of *BCR*, mice have increased susceptibility to septic shock, but normal hematopoiesis.

In CML, each of the two partner genes are disrupted at specific breakpoints and fuse to create the chimeric *BCR/ABL* gene (11). The most common rearrangements give rise to fusion transcripts identified as b2a2 or b3a2, which, in turn, are translated into a 210 kDa protein. Alternative *BCR* breakpoints can be located in minor cluster (m-*BCR*) or micro (μ -*BCR*) cluster (μ -*BCR*) regions and result in fusion transcripts that encode smaller (190 kDa) or larger (230 kDa) products, respectively. Although usually associated with Ph⁺ acute lymphoblastic PiQO leukemia, the protein can also be detected in $\geq 90\%$ of the CML patients from alternative splicing of p210. p230 is instead usually associated with CML patients presenting with an unusual predominance of neutrophils, resembling chronic neutrophilic leukemia. In each of these *BCR/ABL* variants, the *ABL* tyrosine kinase domain autophosphorylates and becomes constitutively activated. Such *BCR/ABL* deregulated tyrosine kinase activity is, in fact, responsible for transformation of the hematopoietic stem cell and maintenance of the leukemic phenotype by recruiting and activating transducing signal pathways (*i.e.*, RAS, RAF, extracellular signal-regulated kinase, c-Jun NH₂-terminal kinase, phosphatidylinositol 3'-kinase, cCbl, CRKL, Janus-activated kinase-signal transducers and activators of transcription, PKC and PLC γ) involved in: (a) enhanced gene transcription (*i.e.*, c-myc, c-Jun, reviewed in Ref. 12); (b) altered mRNA processing, nuclear export, and translation (*i.e.*, bcl-x_L, CAAT/enhancer binding protein α , and p53; reviewed in Ref. 13, 14); and (c) increased or decreased protein stability (*i.e.*, Abi proteins; DNA-PKcs; FUS, and hnRNP A1; reviewed in Ref. 13). This, in turn, leads to enhanced proliferative potential and survival, altered motility and trafficking, and suppression of granulocytic differentiation (1, 13, 15, 16).

Mechanisms of Action of Imatinib Mesylate

BCR/ABL is an ideal target for molecular targeted therapy, because this fusion protein is present in all of the CML cells, is absent from nonmalignant cells, and is necessary and sufficient to induce leukemia. Imatinib mesylate is a 2-phenylaminopyrimidine tyrosine kinase inhibitor with specific activity for *ABL*, platelet derived growth factor receptor, c-kit, and *Alberson-related gene* (17). The pharmacological basis of this interaction has been elucidated by crystallographic studies. Imatinib mesylate binds to the amino acids of the *BCR/ABL* tyrosine kinase ATP binding site and stabilizes the inactive, non-ATP-binding form of *BCR/ABL*, thereby preventing tyrosine autophosphorylation and, in turn, phosphorylation of its substrates. This process ultimately results in "switching-off" the downstream signaling pathways that promote leukemogenesis. Preclinical *in vitro* and *in vivo* data indicated an impressive selective activity of imatinib mesylate on cells expressing *BCR/ABL*, and supported a rapid transition of this compound from the bench to the clinic.

To date, imatinib mesylate has been evaluated in several Phase I and II clinical trials of patients with IFN- α -resistant chronic, accelerated, or BP CML (7, 8, 18–20). From the collective analysis of these studies, imatinib mesylate appears to effectively induce high CHR and cytogenetic response rates with relatively few side effects. In patients with CP CML who have failed IFN- α the CHR was 95%, MCR 60%, and complete cytogenetic remission 46%. Notably, in these patients achievement of MCR at the 3-month time point correlated with improved progression-free survival. In AP and in blast crisis, the CHRs were 34% and 8%, MCRs were 24% and 16%, and complete cytogenetic remissions were 17%, and 7%, respectively. Disease progression was 11% at 18 months for CP, 40% at 12 months for AP, and 80% at 18 months for BP. Finally, preliminary data from an interim analysis of a phase III study of untreated CML patients randomized between imatinib mesylate *versus* IFN- α and ARA-C indicate a significantly better CHR, complete cytogenetic remission, and progression-free survival for the imatinib mesylate group after a median follow-up of 14 months (21). However, a longer follow-up will be necessary to assess whether this compound can also impact on the natural history of the disease and prevent or delay transformation to blast crisis.

Mechanisms of Resistance to Imatinib Mesylate

During disease progression, CML progenitor cells acquire a number of genetic alterations, most probably because of increased genomic instability, that may explain the aggressive phenotype, chemotherapeutic drug resistance, and poor prognosis of CML in BP. Despite the exciting results obtained with imatinib mesylate noted above, CML patients eventually show resistance at a rate of 80% in BP, 40–50% in AP, and 10% in CP post-IFN α failure, at 2 years (19). Identification of the molecular basis of resistance is important, because it could provide insight into disease progression and into the design of novel therapeutic strategies to prevent and overcome treatment resistance.

CML patients with imatinib mesylate resistance can be stratified into those with primary refractory disease, most fre-

quently in accelerated or BPs, and those who relapse after initial response, who are most frequently in CP. On the basis of the presence or absence of BCR/ABL tyrosine kinase activity in leukemia cells, it is also possible to discriminate between cases with BCR/ABL-dependent and -independent mechanisms of imatinib mesylate resistance. Notably, because the BCR/ABL enzymatic activity cannot be easily measured in blood or BM patient samples, levels of phosphorylation of the BCR/ABL substrate CRKL have been used as a surrogate end point for the tyrosine kinase activity (22).

In patients with higher levels of CRKL phosphorylation despite treatment with imatinib mesylate, resistance has been found to result from at least three different BCR/ABL-dependent mechanisms: BCR/ABL gene amplification, BCR/ABL mutations, and high plasma levels of AGP (reviewed in Ref. 23).

The association of BCR/ABL gene amplification with resistance to imatinib mesylate is consistent with the reliance of CML blast crisis cells on BCR/ABL expression/activity for their proliferation and survival, and with the reported enhanced expression of BCR/ABL in these cells (24). Indeed, high levels of BCR/ABL expression, which are detected frequently in CML-blast crisis but not CP cells, appear to be required for suppression of myeloid differentiation and increased resistance to chemotherapy-induced apoptosis of BCR/ABL-expressing cells. Specifically, high levels of BCR/ABL kinase activity are required for hnRNP E2-dependent inhibition of CAAT/enhancer binding protein α , the major regulator of granulocytic differentiation (25), and for the La-dependent enhancement of MDM2 expression, which, in turn, results in functional inactivation of p53 (14), also required for myeloid blastic transformation (26, 27) Although the mechanisms underlying such an increase of BCR/ABL expression are unclear, a double Ph' chromosome is likely to be responsible for the enhanced BCR-ABL levels in some cases.

Other mechanisms of imatinib mesylate resistance involve mutations in the BCR/ABL gene itself. Several different mutations have been detected in at least 13 amino acids of the ATP-binding site or other regions of the tyrosine kinase domain, and the list is growing (23, 28, 29). These mutations usually prevent imatinib mesylate from binding to BCR/ABL, thereby resulting in lack of inhibition of the tyrosine kinase activity. Among these mutations, substitution of a threonine to isoleucine at position 315 of ABL that prevents imatinib mesylate from binding to the ATP-binding domain, is the first described and one of the most frequent (22).

A third mechanism of resistance relies on plasma levels of AGP. It has been shown that AGP binds imatinib mesylate at physiological concentrations *in vitro* and *in vivo*, and blocks the ability of imatinib mesylate to inhibit BCR/ABL kinase activity in a dose-dependent manner (reviewed in Ref. 23). Finally, in patients with primary refractoriness to imatinib mesylate, resistance more often occurs in absence of significant CRKL phosphorylation, suggesting activation of BCR/ABL-independent leukemogenic pathways.

In this issue, Mohamed *et al.* (30) hypothesized that clonal evolution, defined as acquisition of additional cytogenetic abnormalities other than t(9;22)(q34;q11), is a marker for genomic instability, and thereby, in this setting, additional "hits" can occur to activate BCR/ABL-independent leukemogenic mecha-

nisms. Given this premise, CML patients with a more complex karyotype were expected to be more resistant to imatinib mesylate. To test their hypothesis, these authors analyzed 58 BCR/ABL-positive patients with IFN- α -resistant CP ($n = 13$), AP ($n = 24$), or BP ($n = 21$) CML with additional cytogenetic abnormalities who were each treated with imatinib mesylate. Of the 58 patients, 15 (CP = 46%; AP = 25%; BP = 14%) achieved a cytogenetic response, and 12 had a complete cytogenetic remission. With a follow-up of 17–30 months, 7 (12%) remained in complete remission on imatinib mesylate, supporting the notion that a subset of CML patients with t(9;22)(q34;q11) and additional cytogenetic abnormalities can achieve a sustained response. Because the molecular mechanisms of resistance in these patients were not fully evaluated, the contribution of additional cytogenetic abnormalities as an independent predictor of resistance to imatinib mesylate could not be directly addressed. Similar results were also reported in the imatinib mesylate initial studies (18, 20), and more recently by Schoch *et al.* (31). These authors reported on 31 patients with additional chromosomal abnormalities present before the start of imatinib mesylate therapy. Additional cytogenetic abnormalities were less frequent in chronic than other phases of the disease (35.5% versus 68.8%; $P = 0.0008$), and when corrected for the difference in disease stage, they did not influence response to imatinib mesylate. These results have been confirmed recently by a larger study reported by Cortes *et al.* (32) where 498 CML patients in CP or AP were treated with imatinib mesylate. Of the 498, 121 had additional cytogenetic abnormalities. In a multivariate analysis at the 3-month time point, lack of cytogenetic response, but not presence of additional cytogenetic abnormalities, was found to be a negative prognostic factor for survival.

Although a longer follow-up is necessary to draw definitive conclusions, these findings suggest that additional cytogenetic aberrations do not appear to impact on disease response, and, therefore, karyotype analysis should not be used to stratify patients for therapeutic alternatives to imatinib mesylate. Furthermore, despite clonal evolution, the oncogenic potential of BCR/ABL appears to remain the driving force for leukemogenesis, and, therefore, may continue to serve as a therapeutic target. Finally, in patients with additional cytogenetic abnormalities who are resistant to imatinib mesylate, evaluation for levels of CRKL-phosphorylation should be done to sort out the nature of the resistance to this treatment and to understand the interplay between BCR/ABL-dependent and -independent mechanisms in disease progression.

Concluding Remarks

The results obtained with imatinib mesylate to date are truly impressive, but longer follow-up will be necessary to establish whether prevention or delay of blast crisis can be achieved and whether an improved overall survival for the majority of patients with CML can be obtained. It is clear that complete cytogenetic remission is achievable in most patients with CP CML, and that those who do not achieve this important end point between 3 and 6 months are likely to have a poor outcome. However, in patients with complete cytogenetic remission, other challenges remain. In these patients, for instance, molecular remission defined as the absence of BCR/ABL fusion

transcript by RT-PCR is usually not achieved. The reasons for persistent low levels of residual disease after imatinib mesylate and the prognostic significance of these findings are unknown. It is possible that small numbers of mutated and resistant *BCR/ABL*-positive subclones remain essentially unaffected by this treatment, and if a proliferation advantage is subsequently acquired in these cells, they may drive disease recurrence. Therefore, in patients with complete cytogenetic remission, monitoring of the *BCR/ABL* fusion transcript levels by quantitative RT-PCR has been suggested to predict impending relapse, and if rising levels of *BCR/ABL* expression are detected, consideration could be given to alternative strategies including alloBMT.

However, emerging data support the notion that hematologic or cytogenetic remission can be achieved in imatinib-relapsed CP CML patients with higher doses of this agent. Kantarjian *et al.* reported on 54 patients with CML in CP who were initially treated with 400 mg of imatinib mesylate and, subsequently, with a higher dose (*i.e.*, 800 mg) when hematologic or cytogenetic resistance or relapse developed (33). Among 20 patients treated for hematologic resistance or relapse, 13 (65%) achieved CHR without complete cytogenetic remission, and among 34 patients treated for cytogenetic resistance or relapse, 19 (56%) achieved a complete cytogenetic remission or MCR. However, the mechanisms of resistance to standard dose were not evaluated in these patients and, therefore, correlation of these mechanisms with clinical response to the higher doses was not possible. Nevertheless, these data are intriguing and pose the question of whether a higher dose of imatinib mesylate could be used at the time of the initial diagnosis or during molecular relapse to prevent overt leukemia recurrence or blast transformation. Future studies will no doubt address these important issues. Regardless, it is undeniable that imatinib mesylate has changed our approach to CML and paved the way for additional molecular targeted strategies in leukemia.

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