



BCR-ABL kinase is dead; long live the CML stem cell

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Chronic myeloid leukemia (CML) is a hematopoietic disease characterized by expansion of myeloid blood cells. It is caused by the t(9;22) chromosomal translocation that results in the expression of the fusion tyrosine kinase BCR-ABL. Tyrosine kinase inhibitor (TKI) therapy has led to long-term remissions, but patients remain BCR-ABL⁺. There is agreement that TKIs do not kill CML stem cells; however, it is controversial whether this is because of a lack of BCR-ABL kinase inhibition in CML stem cells or because CML stem cells do not require BCR-ABL for survival. In this issue of the *JCI*, Corbin and colleagues provide definitive evidence that BCR-ABL is kinase active in CML stem cells and that TKIs inhibit this kinase activity without affecting CML stem cell survival. Rather, CML stem cells revert to a normal dependence on cytokines for survival and proliferation. These results demonstrate that the CML stem cell is not BCR-ABL addicted and have important implications for developing curative therapeutic approaches to CML.

Chronic myeloid leukemia (CML) is unique among human malignancies in its 1:1 association with the *BCR-ABL* oncogene, which is generated by the hallmark Philadelphia chromosome (Ph) that results from a (9;22)(q34;q11) reciprocal translocation that juxtaposes the Abelson murine leukemia viral oncogene homolog 1 (*ABL*) gene normally located on chromosome 9 with the breakpoint cluster region (*BCR*) gene on chromosome 22. Similarly, it is unique as the poster child for targeted therapeutics, in that the *ABL* kinase inhibitor imatinib has been spectacularly successful at durable restoration of Ph⁺ hematopoiesis and control of progression from CML chronic phase to blast crisis. Recent results from the earliest large trial of imatinib in North America have demonstrated a nearly 90% survival rate after 8 years of follow-up (1, 2). Despite this clinical success, BCR-ABL-containing hematopoietic cells fail to be fully eradicated in the vast majority of patients (3). As assessed by quantitative RT-PCR, *BCR-ABL* transcripts in the blood and/or bone marrow remain measurable and generally stable for years during therapy (3). Importantly, the residual clones are fully capable of restoring leukemic disease, as noted by

expansion of the malignant clone in most patients who discontinue imatinib therapy. Although it has been recently reported that a sustained, leukemia-free state can be maintained in rare individuals after controlled imatinib discontinuation, CML recurrence generally occurs even among patients who previously achieved RT-PCR negative tests for several years (4, 5). Thus, lifelong daily tyrosine kinase inhibitor (TKI) therapy is currently recommended for all patients newly diagnosed with CML. This approach incurs a substantial financial cost, is potentially limited in efficacy by patient compliance, and can be associated with severe side effects and/or secondary medical complications in some patients (6, 7). Hence, the scientific community has embarked on detailed, mechanistic studies of imatinib resistance (i.e., therapeutic failure) and persistence of BCR-ABL-containing hematopoietic cells. In this issue of the *JCI*, Corbin and colleagues provide new insights into this problem (8).

Imatinib resistance is distinct from BCR-ABL persistence

Importantly, the primary reason for early therapeutic failure of imatinib appears to differ from the probable causes of molecular persistence. Most patients whose leukemia initially responds to imatinib but then soon become resistant show emergence of secondary Ph⁺ clones bearing mutations in BCR-ABL itself

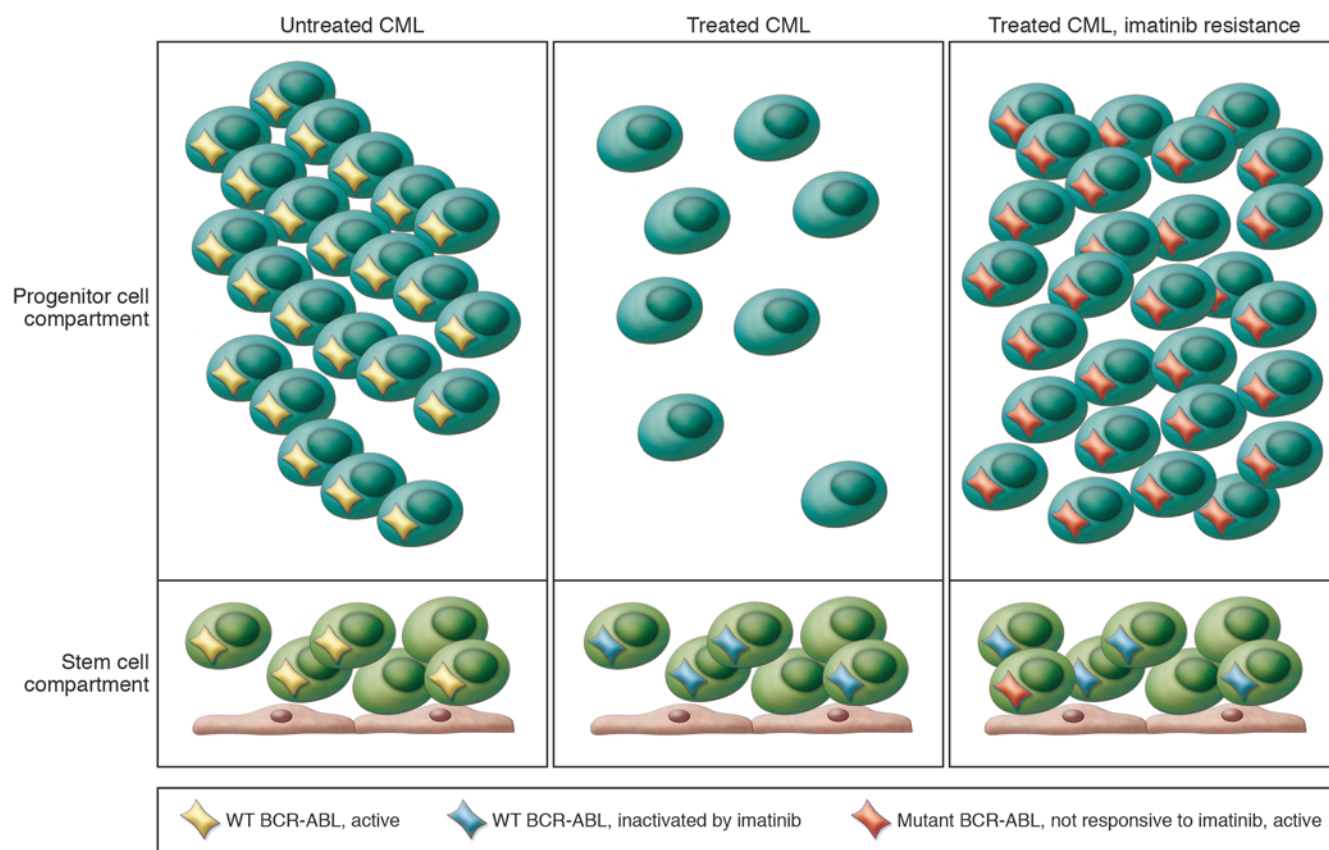
(9, 10). These mutations impair the ability of imatinib to bind to, and thus inhibit, the enzymatic activity of the BCR-ABL kinase. This necessitates therapy with second-generation BCR-ABL inhibitors (e.g., dasatinib and nilotinib) that show more potent activity against native BCR-ABL as well as many of the described imatinib resistance mutations (11–13). Interestingly, low levels of these imatinib-resistant subclones can be demonstrated to precede imatinib therapy (10, 14). Thus, they likely represent clonal diversity of Ph⁺ hematopoiesis at the time of disease diagnosis; and, upon clearance of the major Ph⁺ clone during imatinib therapy, the imatinib-resistant minor clone now exhibits a relative growth advantage and replaces normal hematopoiesis (15). As would be predicted by this model, the outgrowth of imatinib-binding resistance mutations in BCR-ABL occurs early after the initiation of therapy, and their incidence actually appears to decrease with prolonged TKI therapy (16). Thus, imatinib resistance mutations do not explain the common finding of stable persistence of BCR-ABL⁺ cells during years of imatinib therapy, raising the question of whether molecular persistence is BCR-ABL dependent or independent and whether its natural history is sinister or benign.

The CML stem cell and BCR-ABL

This question has led to a series of investigations of the CML stem cell. It is generally agreed that the leukemia-initiating cell in CML is a BCR-ABL⁺ HSC that, per current knowledge, is immunophenotypically indistinguishable from normal HSCs and has the phenotype Lin[−]CD34⁺CD38[−]. The Holyoake lab was the first to demonstrate that CD34⁺ cells from the bone marrow of patients with CML are able to survive in the presence of imatinib and other ABL kinase inhibitors (17). Others have confirmed this observation, but there is substantial controversy as to whether TKIs actually inhibit BCR-ABL kinase in the quiescent stem cell fraction of CML

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**Figure 1**

Summary of BCR-ABL effects in different classes of CML. In untreated patients with chronic phase CML, BCR-ABL is widely expressed and activated in both the stem and progenitor cell compartment. During effective treatment with imatinib or other TKIs, BCR-ABL is inactivated. This allows BCR-ABL⁺ cells to repopulate the progenitor cell compartment while cells that express inactive BCR-ABL persist in the stem cell compartment. The relative level of expression is likely low; the figure is not meant to represent quantitative levels of BCR-ABL persistence. In patients demonstrating imatinib resistance, a mutant BCR-ABL is present in the stem cell compartment that gives rise to progenitor cells with mutant BCR-ABL. The figure represents a steady state, as some reports suggest mutant BCR-ABL may first develop in a progenitor cell and repopulate the stem cell compartment.

cells. Copland and colleagues did not see consistent inhibition of crk-like protein (CrkL) phosphorylation (18), a surrogate marker for BCR-ABL activation, in CD34⁺ cells treated with dasatinib, but Konig and colleagues later showed inhibition of CrkL phosphorylation by both imatinib and the more potent ABL kinase inhibitor nilotinib (19). Importantly, Copland and colleagues used intracellular phospho-flow cytometry to assess CrkL phosphorylation (18), whereas Konig et al. used Western blotting (19). The field has continued to be split on this critical point. Several groups have proposed mechanisms to explain how CML stem cells escape from imatinib and other TKIs, including low intracellular imatinib levels (either caused by reduced expression of imatinib uptake drug transporters or the result of excessive expression of efflux pumps), altered BCR-ABL expression level, and lack of

CML stem cell dependence on BCR-ABL expression (20, 21). The point is critical in moving forward therapeutically since, if BCR-ABL is active in stem cells, we need better ways to inhibit BCR-ABL activity. Alternatively, if it is inhibited, then the CML stem cells persist independently of BCR-ABL activity, suggesting that other approaches to therapy are justified.

In this issue of the *JCI*, Corbin et al. provide a thorough look at BCR-ABL signal transduction in primary CML stem and progenitor populations to determine whether BCR-ABL is indeed a therapeutic target in quiescent cells (8). The authors made extensive use of phospho-flow cytometry and Western blotting to examine signal transduction in stem and progenitor cells from primary patient samples. These experiments were complemented by immunohistochemical staining of sorted stem and progenitor

populations to clarify kinase inhibition for cases in which phospho-flow showed impaired sensitivity. These experiments are of unprecedented quality in this area of investigation and greatly clarify why BCR-ABL is an outstanding target in CML progenitors, but not a rational therapeutic target in CML stem cell populations. The authors unequivocally showed that BCR-ABL was constitutively activated in both CD34⁺CD38⁺CD133⁺ stem and CD34⁺CD38⁺ progenitor populations. They then showed that imatinib potently and equally inhibited BCR-ABL kinase activity in cells from each compartment and that this kinase inhibition occurred at clinically achievable drug concentrations, regardless of whether cells were cycling or quiescent. These biochemical signaling experiments were paired with functional assays to examine the growth, apoptosis, and survival effects of signal inhibition



on each cell type. As expected, imatinib induced potent antiproliferative and proapoptotic effects on CML progenitors in the absence of cytokines. However, CML stem cells treated with imatinib showed growth and survival properties essentially identical to those of normal HSCs, regardless of the cytokine milieu. Finally, long-term culture-initiating cell (LTC-IC) experiments, an assay for stem cells, showed trivial changes in the number of CML stem cells compared with normal HSCs following six weeks of imatinib therapy. The use of second-generation BCR-ABL TKIs for a number of these experiments produced results similar to those observed with imatinib. Taken together, these data demonstrate that BCR-ABL kinase is expressed in CML stem cells and that its enzymatic activity is fully sensitive to inhibition by imatinib (Figure 1). Thus, functionally, imatinib restores normal homeostatic growth control and cytokine dependence seen in nonleukemic HSC populations.

Persistence of CML stem cells is independent of BCR-ABL kinase activation

What do the data generated by Corbin et al. (8) tell us about targeted therapy of CML stem cells in the imatinib era? First, they conclusively confirm that the inability of imatinib to eradicate CML stem cells is a BCR-ABL kinase-independent phenomenon. Although it has been argued that CML progenitor cells are addicted to BCR-ABL signaling for growth and survival, BCR-ABL kinase activity in CML stem cells appears unnecessary for survival (Figure 1). Therefore, if the only defined property that clearly differentiates normal HSCs from CML stem cells is the presence of BCR-ABL, not its function, then efforts to enhance BCR-ABL inhibition in stem cells via the development of more potent inhibitors — blockade of efflux pumps and the like — have little rationale, and this approach should be abandoned.

It is worth asking how this result can be explained. BCR-ABL is a potent, constitutively activated tyrosine kinase, and multiple reports in both cell lines and primary malignant cells demonstrate that BCR-ABL activates signaling pathways. The study by Corbin et al. (8) provides some insight into BCR-ABL signaling in purified CD34⁺ cells, only a subset of which are true HSCs. Recent data have demonstrated the importance of phosphatases in regulating signal transduction in murine HSCs. In the

absence of a more highly refined definition of HSCs in human cells, we would hypothesize that the phosphatases — and other regulatory proteins that keep HSCs quiescent while sitting in a bath of cytokines in the bone marrow — also act to inhibit the effects of BCR-ABL on inducing sustained activation of multiple signaling pathways leading to mitogenesis. In other words, BCR-ABL is always oncogenic, but it is mitogenic only in the proper (progenitor) cell compartment.

This is not to say that targeting the CML stem cell is impossible or should be cast off as a therapeutic goal. Eight-year survival for a previously uniformly fatal disease is a wonderful result of imatinib therapy, but it is definitely not the same as a cure. In this context, the unanswered question is how to target CML stem cells independent of BCR-ABL and without toxicity to normal HSCs. It is likely that drugs that showed activity in CML but were shelved in the wake of the success of imatinib could see renewed interest in the clinic. For example, nonspecific immunotherapy such as interferon could be entertained as a possible adjunct to imatinib, an idea that has been supported by intriguing, although largely anecdotal, reports of long-term disease control using this strategy (4, 22). Clinical trials are ongoing to determine whether this combined approach is indeed curative and/or affords any potential advantage over therapy with imatinib alone. Other approaches with leukemia-specific immunotherapy or arsenicals are also worthy of further examination, as are novel approaches targeting recently identified regulators of CML stemness, such as arachidonate 5-lipoxygenase (Alox5) and Hedgehog (23, 24), or regulators of CML stem cell survival, such as autophagy (25).

Overall, Corbin et al. provide data suggesting that CML is, at its core, a disease of stem cells, but its clinical manifestations during chronic phase occur because of a progenitor cell phenotype (8). Indeed, the stem cell disease is only unmasked once adequate therapy for the progenitor cell fraction has been provided. The data shown in this article demonstrate that for patients being treated with imatinib, BCR-ABL kinase is most likely dead, but the CML stem cell lives on. Time will tell if this will remain the status quo.

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Chemokine antagonism in chronic hepatitis C virus infection

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Immune responses to hepatitis C virus (HCV) fail to clear the virus in most individuals. Why patients who are less likely to clear HCV infection have high plasma levels of CXCL10 (also known as IP-10), a chemokine that directs T cells to sites of infection, has long been unclear. In this issue of the *JCI*, Casrouge and colleagues shed light on this paradox by showing that CXCL10 in the plasma of many HCV patients is enzymatically processed to produce a CXCL10 receptor antagonist. These findings introduce a role for chemokine antagonism during HCV infection and unveil new avenues for improved HCV diagnosis and therapy.

Over 120 million persons worldwide have chronic HCV infection (1), which is a major cause of liver failure and hepatocellular carcinoma (2). Up to one-quarter of persons who are acutely infected with HCV spontaneously clear their infection, and the current standard of care — pegylated IFN- α (peg-IFN- α) and ribavirin — eliminates virus in only about half of those treated (3). This means that a substantial number of patients remain chronically infected with HCV. In these chronically infected individuals, HCV-specific T cells are ineffective at eradicating virus, yet are potent mediators of hepatocellular injury. Evidence presented in this issue of the *JCI* by Casrouge et al. (4) suggests that chemokine antagonism may contribute to this inability to clear HCV infection. Their data (4) also provide an explanation as to why high levels of the chemokine CXCL10 in the plasma or serum of an HCV-infected patient portend a poor response to peg-IFN- α and ribavirin (5–8).

Salient features of chemokines

Chemokines have a central role in inflammation and host defense. These small

(8–17 kDa) cytokine-like molecules act to guide leukocytes along a concentration gradient toward lymphoid organs and sites of inflammation. They also play roles in embryogenesis, angiogenesis, and lymphoid organ development. Chemokines involved in inflammation are displayed on proteoglycans near the site of their production. Chemokines bind to G protein-coupled, seven-transmembrane receptors, of which there are almost twenty. CXCR3, the CXCL10 receptor, is expressed on activated T cells, NK cells, and some B cells (9). In the hepatic sinusoid, leukocyte recognition of chemokines triggers conformational changes in the integrins that they express on their surface, which are then able to mediate binding to endothelial ligands. These steps permit leukocyte transmigration to target tissue (Figure 1) (reviewed in ref. 10).

CXCL10 and liver disease

Among chemokines, CXCL10 plays a central role in liver inflammation, and it is expressed in the HCV-infected liver (11–13). Serum CXCL10 is also elevated during flares in HBV infection (14), in primary biliary cirrhosis, and in rheumatoid arthritis (15). In several independent studies, elevated serum/plasma levels of

CXCL10 predict the failure of IFN- α -based HCV treatment (5–8).

Why a chemoattractant seemingly so potent as CXCL10 is elevated in patients who fail to clear HCV has been paradoxical. One possibility is that CXCL10 is overproduced in a futile attempt to draft pusillanimous T cells into the liver to combat infection. Indeed, chronic HCV infection is often associated with impaired function and reduced breadth of continuously activated, HCV-specific T cells (reviewed in ref. 16). However, in this issue of the *JCI*, data from Casrouge and colleagues suggest that CXCL10 may in fact be dissuading T cells from joining the fight (4).

Casrouge and colleagues performed a multianalyte profiling of patient plasma, confirming that CXCL10 levels are increased in patients that do not respond to anti-HCV therapy compared with those that do (4). They also observed that CXCL10 levels correlated with elevated numbers of circulating CXCR3⁺ cells. It had previously been proposed that the high levels of CXCL10 in patients who do not respond to anti-HCV therapy could act as an antagonist of T cell migration (5). Further, it has been reported that CXCL10 can be processed in vitro by dipeptidyl peptidase IV (DPP4; also known as CD26), which cleaves two amino acid residues from the amino terminus of CXCL10 and turns it into a CXCR3 antagonist (17), and that HCV patients have increased soluble DPP4 activity (18). However, distinguishing full-length from DPP4-processed CXCL10 in clinical samples has not been feasible until now.

After developing reagents to distinguish full-length from DPP4-processed CXCL10, Casrouge and colleagues found

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