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Targeted CML therapy: controlling drug resistance, seeking cure

Thomas O'Hare, Amie S Corbin and Brian J Druker

Targeted cancer therapy with imatinib (Gleevec) has the capability to drive chronic myeloid leukemia (CML) into clinical remission. Some patients, particularly those with advanced disease, develop resistance to imatinib. To counteract this problem, two new BCR–ABL kinase inhibitors for imatinib-refractory disease are currently in clinical trials: the imatinib derivative AMN107 and the dual-specificity SRC/ABL inhibitor dasatinib. Using imatinib to reduce leukemic burden also facilitates the detailed investigation into how the persistence of CML disease depends on BCR–ABL signaling, particularly within the leukemic stem cell compartment. Mathematical models of drug resistance and disease relapse, in addition to experimental systems that recapitulate crucial aspects of advanced disease have deepened our understanding of CML biology. Together, these advances are contributing to a high level of disease control, and might ultimately lead to disease eradication.

Addresses
Howard Hughes Medical Institute, Oregon Health & Science University Cancer Institute, L592, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA

Corresponding author: Druker, Brian J (drukerb@ohsu.edu)

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Introduction

The molecular signature of chronic myeloid leukemia (CML) is the *BCR–ABL* fusion gene, originating from a reciprocal t(9;22) chromosomal translocation in a pluripotent hematopoietic stem cell [1]. The resulting de-regulated tyrosine kinase, BCR–ABL, drives CML [2]. The disease begins with an indolent chronic phase marked by the gradual expansion of myeloid cells with normal differentiation, and then proceeds to advanced phases, including the terminal blastic stage. Disease progression is associated with additional genetic lesions and impaired differentiation [3].

Imatinib (Gleevec, STI571), a relatively selective tyrosine kinase inhibitor that blocks the catalytic activity of BCR–ABL, is the first-line treatment for CML [4]. Most patients treated in the chronic phase of CML achieve a complete cytogenetic remission (Figure 1), as typified by the absence of the t(9;22) translocation in examination of 20 bone marrow metaphase cells. However, BCR–ABL transcripts are detectable by reverse transcriptase PCR (RT-PCR) in ~96% of responding patients, suggesting that this could be a potential pool from which resistance emerges [5]. Molecular persistence has been traced in part to a population of leukemic stem cells. Elucidating the mechanisms by which persistent cells survive imatinib therapy and developing selective strategies to eliminate them are current focal points in CML research [6,7•–9•].

Relapses have occurred in 16% of patients with chronic-phase disease with 42 months of follow-up, but relapses are significantly less frequent in patients who have achieved a complete cytogenetic remission [4,5,10]. By contrast, the majority of patients with advanced phases of disease will relapse on single-agent imatinib therapy, and the main causes of relapse are mutations in the BCR–ABL kinase domain that impair imatinib-binding. Given that similar mechanisms have been observed with other kinase inhibitors, it is likely that acquired resistance will be a common theme of targeted therapy of malignant disease.

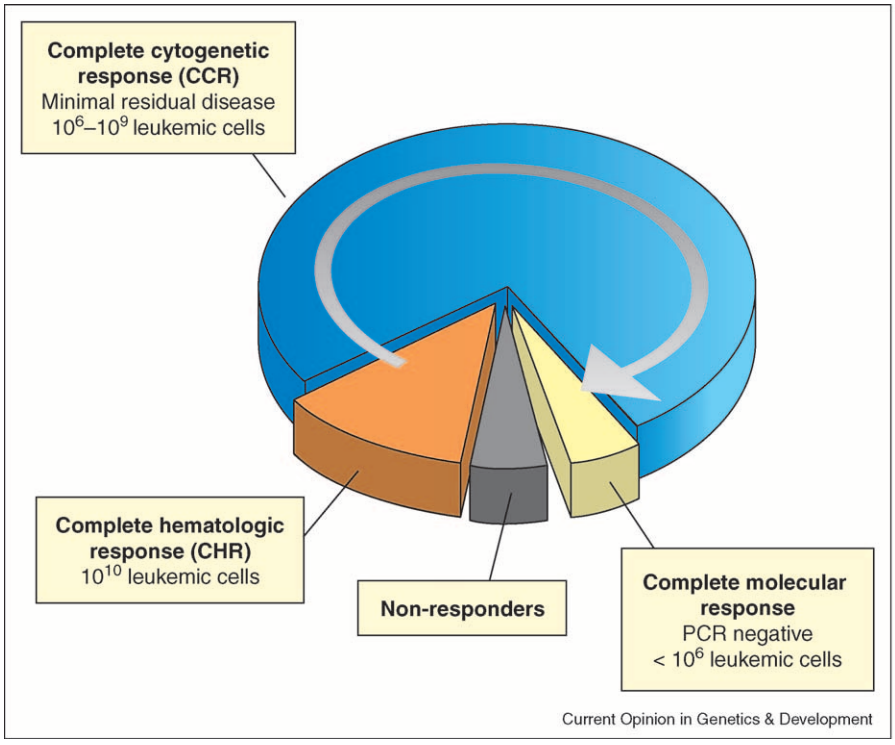
In this article, we review new approaches for controlling disease re-activation caused by acquired drug-resistance. We also highlight the impact of imatinib as a tool for investigating the CML stem cell compartment as it relates to disease persistence and discuss approaches to treating patients with advanced-phase CML.

Leading clinical ABL kinase inhibitors for imatinib-refractory CML

BCR–ABL kinase domain mutations are the leading cause of imatinib resistance, accounting for 60–90% of relapses [11–15]. Although relapse risk remains low for chronic-phase CML patients who achieve a complete cytogenetic remission, relapses are frequent in advanced disease [3]. Several comprehensive reviews detailing imatinib resistance mechanisms are available [12,13,15]. Uncovering BCR–ABL kinase domain mutations as the major mechanism of imatinib-resistant CML has fueled the rapid development of new ABL kinase inhibitors, two of which have advanced to clinical trials: AMN107 and dasatinib (BMS-354825).

AMN107 is a rationally designed imatinib analog with ~30-fold greater potency against BCR–ABL and most

Figure 1



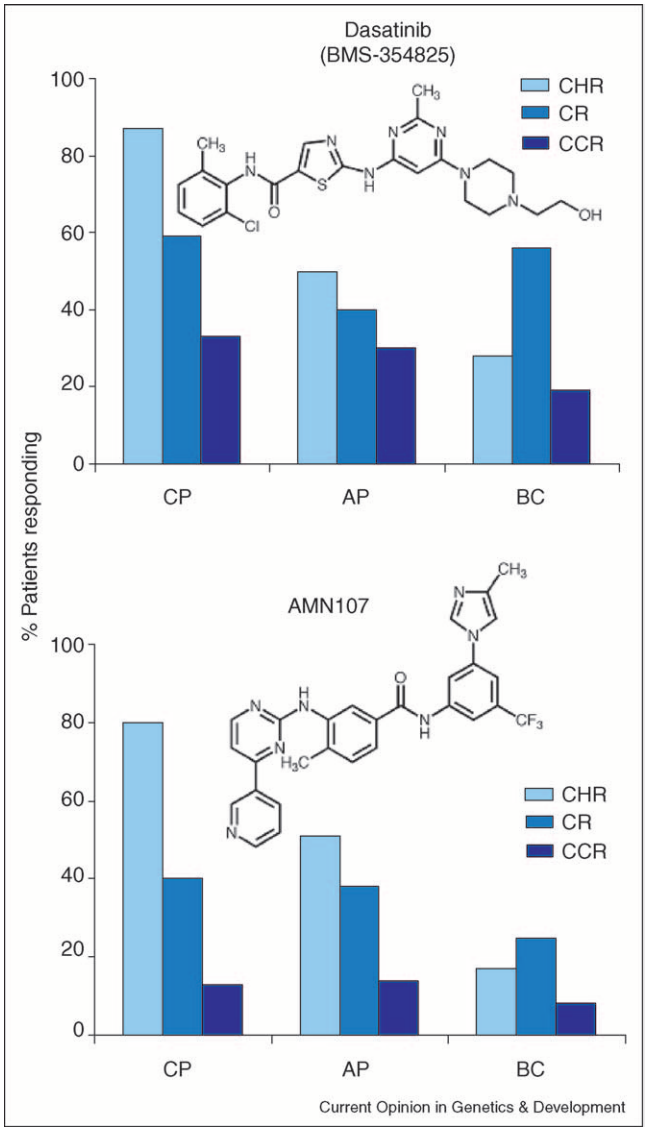
Imatinib-induced reduction of CML disease burden. At diagnosis, chronic-phase CML patients have a disease burden of $>10^{12}$ leukemia cells. Upon imatinib therapy, >95% of newly diagnosed CML patients re-establish normal blood counts, a process termed complete hematologic response (CHR). The curved arrow indicates progressive levels of response among patients achieving CHR. Non-responders to imatinib therapy (~5%) are indicated in grey. Most patients (>85%) experience at least a three-log reduction in CML disease burden after imatinib therapy, to a level categorized as minimal residual disease (MRD). Failure to reach this level is viewed as a poor prognostic indicator. Disease levels below 10^9–10^{10} leukemic cells generally correspond with complete cytogenetic response, defined as the absence of the t(9;22) in either 20 metaphase cells in a bone marrow aspirate or upon sampling of at least 200 cells in a bone marrow aspirate by fluorescence *in situ* hybridization. Molecular responses are common, but few patients (<5%) reach the level of PCR negativity. Thus, almost all responding patients have a residual leukemia burden of $>10^6$–10^7 cells. Measurements of disease burden do not reveal which cell types are susceptible to therapy and which are spared.

imatinib-resistant mutants *in vitro* [16••,17•]. These improvements in affinity are ABL-specific [16••], with the activity of AMN107 against the imatinib-sensitive kinases PDGFR (platelet-derived growth factor) [18] and KIT [19] being similar to that of imatinib (see also Update). AMN107 is currently in phase II clinical trials to determine its effectiveness for treating imatinib-refractory CML, and objective responses are evident in all stages of disease. The percentages of patients with a complete hematologic response are as follows: ~80% of chronic phase patients; ~51% (accelerated phase); and ~17% (myeloid blast phase) (Figure 2).

Dasatinib is a SRC/ABL kinase inhibitor that exhibits ~300-fold higher potency than imatinib against BCR–ABL and most imatinib-resistant BCR–ABL mutants *in vitro* [17•,20••]. Whereas imatinib binds to a unique inactive conformation of the ABL kinase [21], dasatinib is predicted to bind to the active conformation, which is more structurally conserved between ABL and SRC kinases than is the inactive conformation [21]. This enables successful inhibition of most imatinib-resistant mutants; however, it reduces the specificity of the inhibitor and expands the profile of targets to include SRC family members [22,23]. Although dasatinib is the most potent ABL kinase inhibitor identified to date, the true clinical improvement in potency over imatinib will depend on the plasma levels of drug that can be reached in patients. In phase I clinical trials, the percentages of patients who attained a complete hematologic response are as follows: ~87% (chronic phase); ~50% (accelerated phase); and ~28% (myeloid blast phase) (Figure 2). Importantly, due to different inclusion criteria and shorter follow-up in the AMN107 cohort, the data from the two studies are not directly comparable. Also, both of these studies are ongoing and are not yet at a stage that enables direct comparison with results from completed clinical trials for imatinib [4,5,10].

These two new ABL kinase inhibitors have been developed and taken into clinical trials within an impressive time-frame. Barring serious side effects, future studies

Figure 2



Early clinical trials results for dasatinib (upper panel) or AMN107 (lower panel) treatment of imatinib-refractory and intolerant CML patients. Results from the two trials are not directly comparable, as a result of shorter follow-up in the AMN107 cohort and slight differences in enrolment criteria. Abbreviations: AP, advanced phase; BC, myeloid blast crisis; CCR, complete cytogenetic response; CHR, complete hematologic response; CP, chronic phase; CR, partial cytogenetic response. Data sources: Dasatinib [54,55]; AMN107 [56,57].

will focus on expanded clinical uses for these drugs in patients with CML. Given suggestions that higher-dose imatinib therapy might achieve higher rates of molecular response to imatinib, it will be of interest to see if this is the case with these more potent inhibitors. However, emerging data suggest that even more-potent inhibitors are not capable of eliminating all CML stem cells [24]. As with imatinib, it is likely that patients with advanced-phase disease will develop resistance. With this knowledge, it is worth preparing for this possibility.

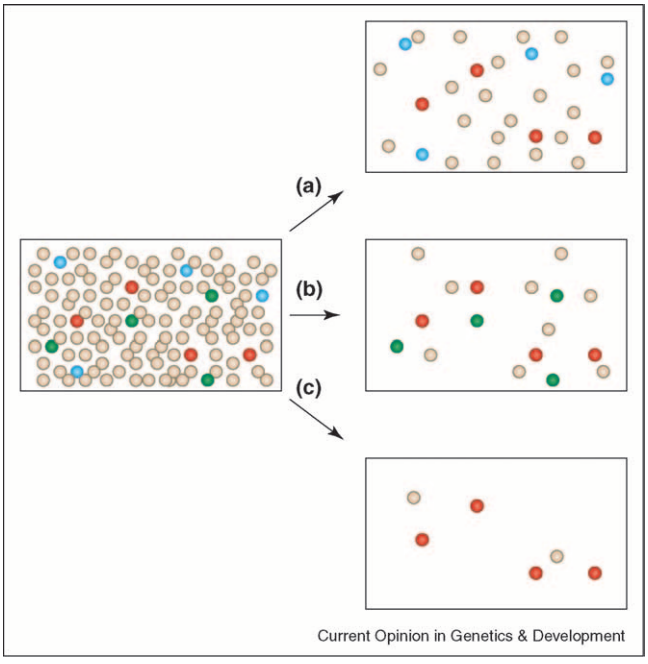
Addressing clinical resistance to new ABL kinase inhibitors

Clinical experience with imatinib demonstrates that drug exposure can result in selection for outgrowth of drug-resistant CML cells. An *in vitro* saturation mutagenesis screen [25] and a cell-based screening strategy [26•] identified BCR–ABL point mutations implicated in clinical resistance to imatinib. In the saturation mutagenesis method, random point mutations are introduced into *BCR–ABL* by propagation of the target construct in an *Escherichia coli* strain deficient in three major pathways of DNA repair. The mutated constructs are used to transfect Ba–F3 cells, and point mutants conferring drug resistance are selected in the presence of graded concentrations of imatinib. In the cell-based screening method, Ba–F3 cells stably expressing BCR–ABL are cultured at high density in the presence of graded concentrations of inhibitor corresponding to between 2.5 and 20 times the cellular IC_{50} value. Single colonies surviving under these conditions are picked, expanded and analyzed for kinase domain mutations as well as other mechanisms of resistance. Similar strategies can be used to predict resistance mutation profiles that are likely to emerge during treatment with either AMN107 or dasatinib.

Screening for BCR–ABL mutations that confer resistance to dasatinib revealed that three mutations, T315I, T315A and F317V, accounted for >90% of the recovered clones [27]. Among these, BCR–ABL with T315A and F317V mutations retain considerable sensitivity to imatinib [27]. These findings suggest that treatment with a cocktail of two or more ABL kinase inhibitors could suppress a broader profile of resistant mutants and eliminate a higher proportion of leukemic cells than does single-agent therapy (Figure 3) [27,28]. Although the tolerability of such treatment regimens must be addressed in clinical trials, the availability of two new ABL kinase inhibitors with predicted mutational profiles distinct from one another and from imatinib might minimize acquired drug-resistance and prolong responses.

A general predictive model that directly addresses resistance to targeted cancer therapy invokes three pretreatment parameters: tumor cell turnover rate, mutation rate, and effective tumor size [29•]. When applied to CML, the prediction emerges that combining three targeted drugs with different specificities might overcome drug resistance in this cancer. If one equates 'specificities' with mutation profiles rather than with distinct molecular targets, ABL kinase inhibitor cocktails, in principle, meet this criterion for overcoming drug resistance.

Figure 3



Comparison of single-agent and two-agent ABL kinase inhibitor therapy. A hypothetical scenario in which leukemic cells express BCR–ABL (beige) or one of three BCR–ABL mutant proteins (blue, green or red) with kinase domain mutations conferring resistance to drug A, drug B or both drugs, respectively. **(a)** Single-agent therapy with drug A. **(b)** Single-agent therapy with drug B. **(c)** Combined therapy with drugs A and B. Potential benefits of ABL kinase inhibitor cocktail therapy include reduction in overall number of leukemic cells and elimination of a wider range of cells expressing drug-resistant variants of BCR–ABL. The presence of residual CML cells provides a possible mechanism for eventual relapse. Expansion of cells colored in red is possible under all three conditions and applies most notably to cells expressing BCR–ABL(T315I), for which no clinical inhibitor has yet been identified.

The unsolved problem of BCR–ABL(T315I)

The T315I mutation, accounting for 10–15% of clinically observed mutations, confers complete resistance to all clinically available kinase inhibitors [12,13,15]. Structural analysis predicts that the T315I mutation eliminates a crucial hydrogen-bonding interaction required for high-affinity imatinib-binding and alters adversely the topology of the ATP-binding pocket [21]. Despite the pressing need for a clinically effective BCR–ABL(T315I) inhibitor, relatively few pre-clinical candidates have been reported [30,31]. A potential pitfall might be the tendency to screen initially for ABL kinase inhibition rather than for ABL(T315I)-inhibition.

An alternative approach is to target other regions of BCR–ABL. For example, ON012380, a putative substrate-competitive inhibitor of BCR–ABL exhibits low nanomolar activity against imatinib-resistant BCR–ABL mutants, including T315I [32]. Studies to define the precise binding site of ON012380 in addition to its anticipated mutation pattern will be highly informative. Other regions of BCR–ABL that could be exploited for therapeutic intervention include oligomerization and SH3 (SRC-homology 3) domains [33], the myristoyl-binding pocket [34], and the F-actin binding domain, a determinant of BCR–ABL interactions with cytoskeletal components [35].

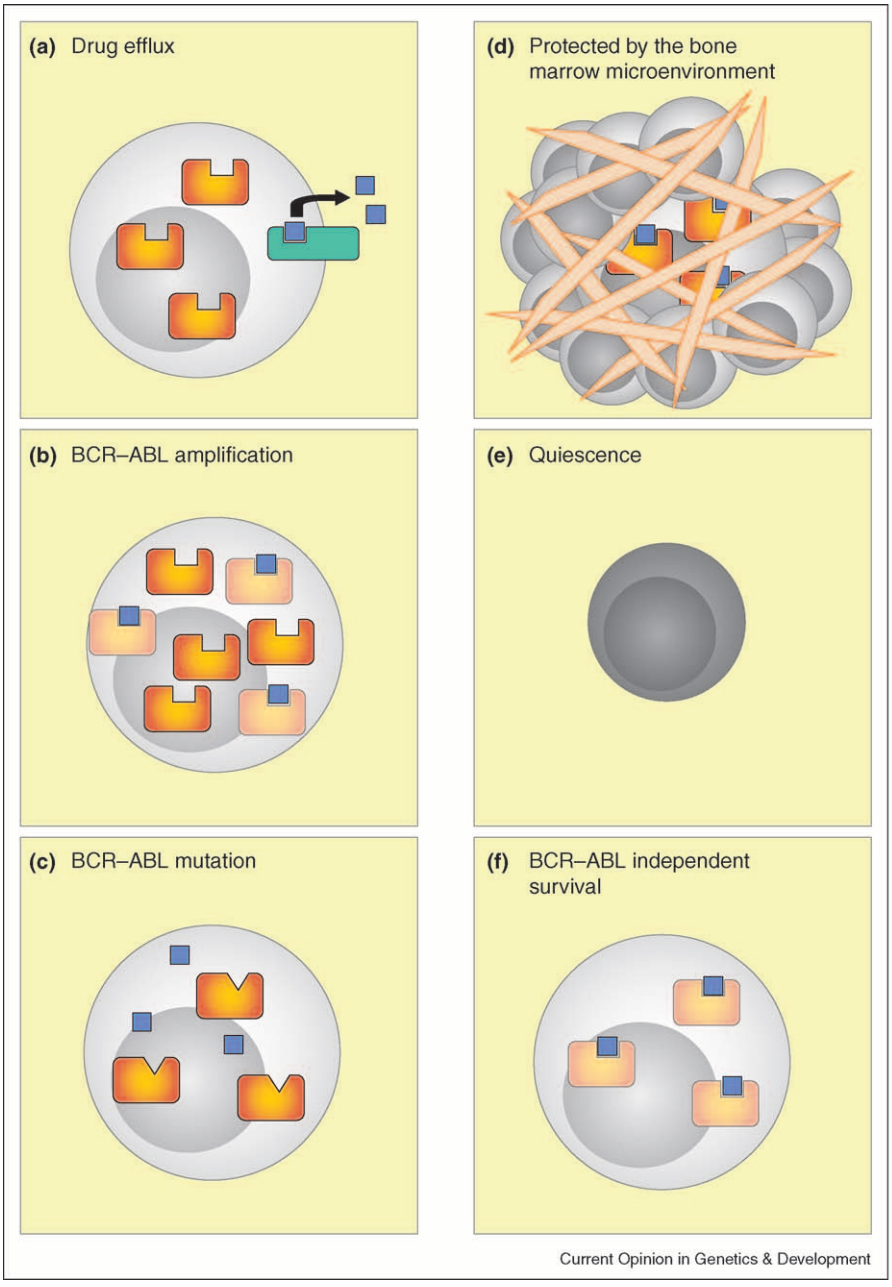
Can imatinib target leukemic stem cells?

Modeling the kinetics of imatinib response [36•] in chronic phase CML patients quantitatively validates an emerging consensus that imatinib inhibits the production of differentiated leukemic cells but does not deplete leukemic stem cells. The role of malignant stem cells is firmly established in hematopoietic cancers [7•], and it is clear that leukemic stem cells encompass a hierarchy of developmental stages [7•,37•,38]. An obstacle to therapeutic elimination of leukemic stem cells is the need to preserve normal hematopoietic stem cells, which have many fundamental properties in common with leukemic stem cells. Establishing the expression pattern of BCR–ABL in primitive cells and whether or not BCR–ABL function is critical to leukemic stem cell survival will guide the development of strategies to eliminate leukemic stem cells in CML.

Numerous specific hypotheses could individually or collectively explain how primitive, BCR–ABL-positive cells avoid the pro-apoptotic effects of imatinib. The proposed mechanisms can be separated into two categories: those for which targeting BCR–ABL might still be therapeutically effective (i.e. drug efflux [9•,39], BCR–ABL target amplification [40] and kinase domain mutations [24]); and those for which BCR–ABL is not an appropriate target, including protection through the microenvironment [41], stem cell quiescence [6,8•], and BCR–ABL independence (Figure 4) [42]. Quiescent cell survival might also be attributable to inherent traits (e.g. drug efflux) as opposed to quiescence itself representing a direct persistence mechanism. Recently, it was demonstrated that imatinib is a substrate for human organic cation transporter 1 (hOCT1) [43], and a requirement for expression of appropriate influx transporters might represent an additional mechanism of persistence.

The presence of BCR–ABL mutations in samples from complete cytogenetic remission patients has been documented [24], but the link to disease persistence is tenuous at present [44]. If BCR–ABL mutations are the primary mechanism of disease persistence, AMN107 or dasatinib might be effective at eliminating these cells. One possibility is that, although leukemic stem cells serve as the earliest repository of the *BCR–ABL* molecular abnormality, they do not require BCR–ABL signaling for survival. In this scenario, more-potent ABL kinase inhibitors would also be ineffective, and alternative strategies would be necessary. Preferential induction of apoptosis in leu-

Figure 4



Potential mechanisms of disease persistence on imatinib therapy. BCR–ABL is shown in orange; imatinib is in blue. **(a)** Enhanced drug efflux by ABCG2 (ATPase-binding cassette G2) and/or other transporters (green). **(b)** BCR–ABL target amplification by increased BCR–ABL transcript levels or gene amplification; insufficient concentration of inhibitor to completely shutdown kinase activity. **(c)** BCR–ABL kinase domain mutations render persistent cells insensitive to imatinib. **(d)** Protection within bone marrow microenvironment; stromal cells surround persistent cells. **(e)** Quiescent, non-cycling cells in deep G_0 are impervious to the pro-apoptotic effects of imatinib. **(f)** BCR–ABL is efficiently targeted, but BCR–ABL kinase activity is dispensable for persistent cell survival.

kemic stem cells [45], and the use of vaccines and immunotherapy [6,46] are being pursued with preliminary success. At a basic research level, dissecting the molecular details of engraftment and mobilization provides a basis for identifying new targets for therapeutic intervention [47,48].

Targeted therapy in advanced disease

Responses to imatinib in blast crisis can be dramatic but are generally short-lived. The molecular events that drive disease progression remain incompletely understood [3], and it is not clear whether more-potent ABL kinase inhibitors will improve the prognosis for advanced-phase

patients. Loss of p53 function might be important in CML disease progression, as demonstrated by genetic inactivation of p53 in ~30% of CML blast-crisis cases. MDM2 (mouse double minute 2) is a negative regulator of p53, and the finding that BCR–ABL activates translation of MDM2 mRNA provides a possible mechanism for functional inhibition of the p53 pathway [49]. The MDM2 pathway might, therefore, be an appropriate therapeutic target for treatment of advanced CML [50].

Granulocyte-macrophage progenitors from patients with CML in blast crisis were recently reported to exhibit self-renewal activity *in vitro*, possibly through activation of β-catenin [51•]. Follow-up studies are required to assess whether this cadre of committed progenitor cells can initiate disease in animal models. Two additional studies support the possibility that committed progenitor cells can acquire self-renewal capacities in the context of acute leukemias [52,53] Together, these provocative findings suggest that strategies designed to eliminate committed progenitor cells imbued with leukemic stem cell-like properties might be effective in controlling advanced leukemias.

Conclusions

Well into the first decade of the imatinib era — and amidst tremendous gains — problems remain: acquired drug resistance, persistence at the level of minimal residual disease, and limited therapeutic options for treating advanced disease. Imatinib targets malignant cells that strictly depend on sustained BCR–ABL kinase activity for survival. Much remains to be unraveled about the leukemic cells at the two extremes of disease: stem cells in minimal residual disease, and blasts in advanced disease.

CML treatment is not yet directed to the root of the disease but, instead, at its most vulnerable point, the BCR–ABL kinase. ABL kinase inhibitors, possibly as cocktails or in combination with other inhibitors, still represent the best therapeutic option for establishing and maintaining clinical remissions. Although cure is the ultimate goal of CML therapy, we accept that the more immediately accessible frontier is to reach a residual disease threshold below which relapses are rare. For many CML patients, this might be as near to a cure as we can or need to get.

Update

AMN107 has been identified recently as an effective inhibitor of the fusion tyrosine kinases TEL–PDGFRβ and FIP1L1–PDGFRα, which cause chronic myelomonocytic leukemia and hypereosinophilic syndrome, respectively [58•].

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