Targeted CML therapy: controlling drug resistance, seeking cure
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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.

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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
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
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 IC₅₀ 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 pre-treatment 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.
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 leukemia stem cells is firmly established in hematopoietic cancers [7*,37*,38], 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.

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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 inactivation 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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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

7. Huntly BJ, Gilliland DG: Leukaemia stem cells and the evolution of cancer-stem-cell research. Nat Rev Cancer 2005, 5:311-321. This review begins with an historical account of how we came to our current view of leukemic stem cells then covers differences among leukemic stem cells, normal hematopoietic stem cells and committed progenitors that have acquired self-renewal capabilities. Possible approaches for selective targeting of leukemic stem cells are addressed.

This first report describing the new imatinib family member AMN107 includes a crystallographic analysis of AMN107 in complex with the imatinib-resistant mutant ABL(M351T). Comparison of this structure with the imatinib–ABL complex provides a structural rationale for the improved ABL-binding affinity of AMN107.


This study uses cellular and biochemical assays to directly compare the effectiveness of imatinib, AMN107 and dasatinib (BMS-354825) against a broad panel of imatinib-resistant BCR–ABL mutants.


This study introduces the orally bio-available SRC/ABL kinase inhibitor dasatinib (BMS-354825) and demonstrates in vivo activity against BCR–ABL and the imatinib-resistant mutant BCR–ABL (M351T) in a mouse model of disease.


This study introduces a cell-based method of identifying drug resistant BCR–ABL mutants. Ba-3 cells transfected with wild type BCR–ABL were grown at high density in the presence of imatinib or the SRC/ABL inhibitor PD166326, and sub-lines were established from surviving colonies. In contrast to the method described by Azam et al. [25], mutations were confined to the BCR–ABL kinase domain.


A mathematical model that uses three measurable disease parameters to predict whether combination therapy is likely to reduce the incidence of drug resistance compared with the incidence after single-agent therapy. Cancers with low rates of tumor cell turnover and/or low tumor mutation rates fare better in this analysis than cancers with high turnover rates and/or high tumor mutation rates. For example, the model predicts that resistance in CML may be controllable with three drugs, whereas others cancers require ten or more non-cross-resistant drugs.


The decline of BCR-ABL transcript levels of chronic phase CML patients during the first year of imatinib therapy was analyzed using a quantitative model of disease dynamics. Under successful imatinib therapy, differentiated leukemic cells survive for ~20 days, and more-primitive leukemic progenitors have a ~sixfold longer lifespan. This model is consistent with the putative inability of imatinib to deplete leukemic stem cells.


Meticulous tracking of individual leukemic stem cells following serial transplantation of NOD-SCID (non-obese diabetic severe combined immunodeficient) mice with AML leukemia cells revealed that leukemic stem cells comprise a functionally heterogeneous class and exhibit a range of self-renewal capacities, in close analogy to normal hematopoietic stem cells.


40. Xiaoyan J, Zhao Y, Chan WY, Pang E, Eaves A, Eaves C: Leukemic stem cells of chronic phase CML patients consistently display very high BCR-ABL transcript levels and reduced responsiveness to imatinib mesylate in addition to generating a rare subset that produce imatinib mesylate resistant differentiated progeny. Blood 2004, 104:204a.


In *vivo*, AMN107 inhibited proliferation of Ba–F3 cells transformed with TEL–PDGFRα, imatinib-resistant mutant TEL–PDGFRα T681I or FIP1L1–PDGFRα (IC50 < 25 nm in each case). *In vivo* bone marrow transplantation assays demonstrated that AMN107 was an effective treatment for myelo-proliferative disease induced by either TEL–PDGFRα or FIP1L1–PDGFRα.