Disabling Abl—Perspectives on Abl kinase regulation and cancer therapeutics

Pharmacologic inhibition of the Bcr-Abl tyrosine kinase in human chronic myeloid leukemia leads to dramatic clinical responses, but relapses occur in advanced stage patients. New findings about Abl kinase domain regulation provide insight into novel strategies for targeted therapy.

The clinical success of the Abl kinase inhibitor STI-571 (Gleevec/imatinib) in the treatment of chronic myeloid leukemia (CML) has focused renewed enthusiasm toward understanding the pharmacology of kinase inhibition. Virtually all the kinase inhibitors currently in clinical testing were discovered through the process of empiric high-throughput screening of large chemical libraries against kinase targets rather than through rationale design of drugs based on knowledge of kinase domain structure. However, there is great optimism that crystallographic data of kinases in various states of activation will lead to structure-based drug design. A recent article in *Cell* by Superti-Furga and colleagues provides compelling evidence that the c-Abl kinase is negatively regulated by the N terminus of the wild-type protein, which is deleted when fused to Bcr (Pluk et al., 2002). While this model must be confirmed by crystallographic studies, this mode of kinase regulation could have implications for the design of future kinase inhibitor drugs.

Solutions of the Src and Hck structures provided the first evidence that this family of kinases is regulated by a complex set of intramolecular interactions between the SH2, SH3, and catalytic domains. One unanticipated finding was that the linker region between the SH2 and catalytic domains forms a polyproline helix which binds the SH3 domain, bringing it into close proximity with the catalytic domain where it exerts an inhibitory function (Sicheri et al., 1997; Xu et al., 1997). Although similar structural data for Abl is not yet available, the homology between Abl and Src kinases as well as mutational studies suggest a similar mode of regulation (Barila and Superti-Furga, 1998). A critical aspect of Src kinase regulation is an intramolecular interaction between the SH2 domain and a phosphotyrosine residue in the C terminus. This interaction plays a negative regulatory role, in part, by positioning the SH3 domain in a proper configuration for inhibition of the catalytic domain. This design permits Src regulation at multiple levels—through phosphorylation/dephosphorylation of the C-terminal tyrosine residue as well as through competition between intramolecular and external ligands for SH2/SH3 domain binding.

A long-standing puzzle surrounding Abl regulation stems from the fact that its C terminus does not contain an analogous regulatory tyrosine residue that could function to bind the Abl SH2 domain. Without this analogous structure, how could the inhibitory SH3-catalytic domain interaction be maintained? Superti-Furga and colleagues provide strong evidence that the N terminus of c-Abl serves this function by forming a "cap" structure, through direct interaction with the catalytic domain and SH2/SH3 domains (Pluk et al., 2002) (Figure 1). In addition to providing an attractive solution to the puzzle over differences in Src versus Abl regulation, the new data will force a reexamination of the function of several Abl binding proteins previously proposed to function as cellular inhibitors of Abl.

The Superti-Furga paper raises some fascinating issues regarding the biology of tyrosine kinase regulation. But does it have implications for the treatment of diseases such as CML, whose pathogenesis is dependent on Abl kinase activation through fusion to Bcr? This question is of immediate interest based on the remarkable success of the Abl kinase inhibitor STI-571 in treating patients with various stages of CML. STI-571 was originally isolated in a high throughput screen for small molecule inhibitors of the PDGF receptor tyrosine kinase (Buchdunger et al., 1995), and subsequently shown to have potent inhibitory activity against two other tyrosine kinases, Abl and c-Kit. The large body of clinical and experimental evidence that the Bcr-Abl fusion protein is the initiating oncogenic event in CML (reviewed in Sawyers, 1999) led to preclinical studies showing activity of STI-571 against Bcr-Abl expressing tumor cell lines and bone marrow cells isolated from patients with CML (Druker et al., 1996). A phase I clinical trial showed a 98% hematologic response rate in CML patients in the chronic phase of the disease and a 60%–70% hematologic response rate in the end stage of the dis-

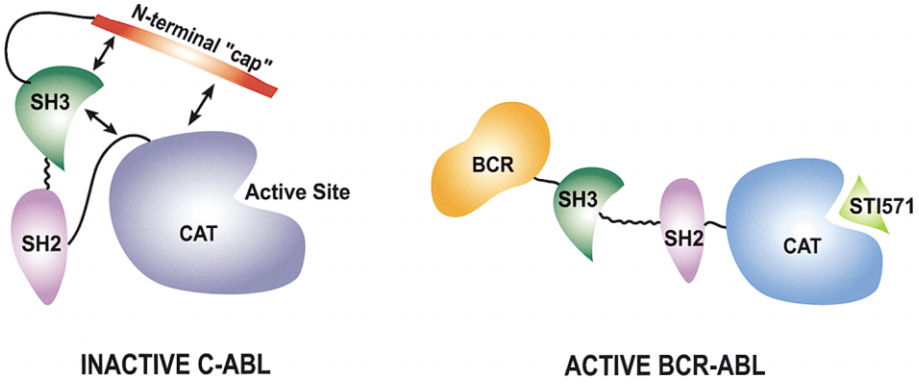

Figure 1. The putative structure of Abl in the regulated, inactive conformation is shown on the left, with the SH3 domain exerting an inhibitory function on the catalytic domain (CAT) by binding to the SH2-CAT linker region. The N-terminal cap (red) locks the SH3 domain in this configuration by binding to it as well as the CAT domain. In the Bcr-Abl fusion protein (right panel), the N-terminal cap is removed and the kinase domain remains unregulated.

ease, termed blast crisis (Druker et al., 2001a, 2001b). STI-571 was approved for clinical use by the US Food and Drug Administration in May, 2001, based on similar results in phase II studies.

One of the remarkable surprises from these clinical trials was the high response rate in CML patients with blast crisis, indicating that these tumor cells depend on Bcr-Abl despite the presence of multiple additional oncogenic abnormalities. Unfortunately, these patients relapse despite continued treatment with STI-571. Recent investigations into mechanisms of STI-571 resistance shows that Bcr-Abl signaling, which is inhibited in leukemia cells when patients are responding to STI-571 treatment, is reactivated at the time of relapse (Gorre et al., 2001). It seems, therefore, that blast crisis cells rely on the Bcr-Abl signal transduction pathway even at the very latest stages of disease. To date, the primary mechanisms responsible for Bcr-Abl reactivation are kinase domain mutations which impair binding of STI-571 to the active site in the catalytic domain or amplification of the Bcr-Abl gene (Gorre et al., 2001).

Structural data of the Abl kinase domain bound to STI-571 (Schindler et al., 2000) has been critical to understanding how these mutations detected in relapsed CML patients confer drug resistance. The best documented example is a threonine-to-isoleucine change reported by several groups (Gorre et al., 2001; summarized in Sawyers, 2001). This threonine residue resides on the surface of the drug binding pocket and forms a hydrogen bond with STI-571. In the mutant Bcr-Abl allele, the bulkier isoleucine substitution prevents STI-571 binding due to steric hindrance at the drug binding pocket and inability to form the hydrogen bond. Because this threonine is conserved in many tyrosine kinases, it might be anticipated to play a more general role in mechanisms of kinase inhibition by other small molecule inhibitors as well as in mechanisms of potential drug resistance. Another important insight from the Abl/STI-571 structure is a better understanding of how certain inhibitors can show differential activity against kinases that appear quite similar by amino acid sequence, particularly at the ATP binding site. STI-571 binds to Abl when the kinase domain is in the "off" configuration (Schindler et al., 2000), which is structurally quite distinct from the "off" configuration of Src. This structural difference between Abl and Src provides a compelling explanation for why STI-571 shows inhibitory activity only against Abl. On the other hand, kinase inhibitors such as PP-1 have activity against both Src and Abl (Liu et al., 1999), perhaps because these compounds bind the kinases in an "active" configuration where they are structurally more similar.

Could the new insights about Abl kinase regulation provided by the Superti-Furga paper help design better kinase inhibitors for CML? To address this possibility, it is critical to understand the mechanism of Abl kinase activation in the Bcr-Abl fusion protein. The prevailing model is that the coiled-coil dimerization motif in Bcr serves as a gain-of-function mutation that leads to constitutive Abl kinase activation. The Superti-Furga paper raises the intriguing possibility that the fusion protein, which lacks the inhibitory N terminus of Abl, is activated by a loss-of-function mechanism (Figure 1). I can envision two potential consequences for clinical medicine. First, there are many patients with CML-like hematologic disorders that defy molecular classification because they lack the Bcr-Abl fusion protein (this disease is often called Philadelphia chromosome negative CML). Fusions of Abl or the Abl-related protein Arg to the Ets-like protein Tel (also called ETV6) have been reported in rare cases (Papadopoulos et al., 1995; Cazzaniga et al., 1999), and the mechanism of Abl/Arg activation in these fusions appears similar to Bcr-Abl since Tel also contains a dimerization motif (Golub et al., 1996). It will be important to know if any cases of Philadelphia chromosome negative CML might be explained by N-terminal mutations that lead to Abl activation. A second clinical implication of the Superti-Furga model is that regulation of Abl kinase activity might be restored in CML by forcing the SH2/SH3 domain and linker structure back into a conformation that inhibits the catalytic domain. The authors provide some evidence that this may be possible by showing inhibitory effects of the N-terminal cap on Abl kinase activity when provided in *trans*. Analogous experiments with Bcr-Abl may prove more challenging, since reconstitution of the N-terminal cap in *cis* with Bcr-Abl (through creation of a Bcr/N-cap/Abl fusion) failed to inhibit kinase activity unless the dimerization motif in Bcr was impaired. This result should not discourage further investigation because it may be possible to target Bcr independently, as the crystal structure of this dimerization motif has recently been solved (Zhao et al., 2002). In a recent episode of the popular television show West Wing about life in the White House, President Bartlett considered a campaign to eliminate cancer in ten years, based on new evidence that signal transduction inhibitors (called STI) can cure cancer. Hopefully this fictional episode will one day become a nonfiction documentary.

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Functional genomics and the breast cancer problem

The clinical treatment of primary breast cancers has been greatly complicated by the inability to accurately predict which tumors will eventually become invasive and metastatic and which will remain localized and indolent. Lacking the ability to discriminate between these two classes of breast cancer patients, oncologists often apply aggressive adjuvant therapy to women in both groups. However, the use of functional genomics analysis has now made it possible to assemble a set of gene markers, the expression of which enables one to predict, with reasonably high accuracy, whether or not the patient will relapse or remain tumor-free five years after initial diagnosis and treatment.

The application of functional genomics to the analysis of breast cancer samples holds great promise in producing a substantial advance in breast cancer prognostication. In a recent issue of *Nature,* van't Veer et al. describe their ability to determine with substantial accuracy whether a young node-negative patient will or will not show progressive disease within five years time (van 't Veer et al., 2002).

This work highlights a painful disparity in current clinical practice: oncologists are able to detect tumors of ever smaller size (current avg.: ~1.5 cm diameter) in the breast, but their ability to exploit the diagnostic parameters associated with these relatively small tumors in order to predict eventual outcome has lagged far behind.

The oncologic territory has changed dramatically over the past two decades. With improving public awareness and detection techniques, as many as 60%–70% of breast cancer patients currently present clinically at an early stage while still being node-negative. That is, the axillary lymph nodes, which drain much of the mammary gland, are found to be free of detectable cancer cells through light microscopy.

Since the 10-year recurrence rate in node-positive patients is approximately 70%, the standard practice is to administer systemic adjuvant therapy to all patients in this group. This therapy involves anti-estrogen treatment (in the case of estrogen receptor-positive tumors), Herceptin (in the case of HER2/*neu*-positive tumors), and often cytotoxic drugs.

The treatment decision for node-negative patients, however, is not as straightforward. Approximately 70%–80% of the node-negative group will survive breast cancer without additional treatment beyond surgical resection of the initially detected growth**.** Hence, many argue that adjuvant systemic therapy, which can have significant toxicities, should not be undertaken routinely, since one risks harming many for the benefit of a few (Harris et al., 2000; Bland et al., 1998).

The dramatic shift in the clinical status of women at the time of initial presentation in the clinic now creates a substantial and still-to-be-solved problem, because many of the standard pathologic prognostic factors currently used were developed in an earlier era when a majority of the patients presented with larger (>2 cm diameter) tumors and were node-positive.

Among the many studies that have examined prognostic factors for breast cancer, only a few have specifically focused on node-negative patients (Mirza et al., 2002). Moreover, standard prognostic factors such as tumor size, histological type, grade of differentiation, and hormone receptor status were found to have limited value in predicting which node-negative patients would relapse. As a result, physicians have very few tools available for determining which node-negative patients should receive adjuvant therapy.

The landscape has also changed in yet another respect. Some of the traditional markers (e.g., estrogen receptor, ER) have been useful not only to predict outcome but also to inform therapy, since anti-ER agents such as tamoxifen have been available and found useful for improving short-term survival. Now, with the advent of novel therapies (e.g., Herceptin), other markers, such as Her2/*neu* expression, gain in importance because of their role in conferring responsiveness to these newly developed agents.

This complex picture reveals the disease of breast cancer to be a rapidly moving target, where the perceived relevance of widely used diagnostic and prognostic parameters may change dramatically over a period of several years.

The mysteries of breast cancer epidemiology

The number of diagnosed breast cancers in the Western world has increased substantially over the past half century. For example, in 1990, an estimated 150,000 new cases were reported in the United States while six years later, the number had increased to 185,000. During the same time period, estimated deaths due to breast cancer remained constant (~44,000) (Harris et al., 2000). However, there is some hope on the horizon: over the past several years, age-adjusted mortality of breast cancer has actually declined slightly (~1.7% per