keeping with current enthusiasm around proteomics technologies. But perhaps this new spin will reawaken flow cytometry's proven record as a robust means of quantitatively assessing protein expression at the individual cell level. Third, the authors demonstrate that this approach can be taken not only with established cell lines, but also with primary leukemic blasts. Indeed, a significant amount of heterogeneity in cellular response was observed within individual patient samples. Whether this heterogeneity will prove to be clinically important remains to be determined.

To be sure, the study is limited by the small numbers of analytes (e.g., Stat proteins) and small number of perturbations (cytokine stimulations). But, the proof of principle is established that classification based on dynamic response to perturbation is feasible and informative. As higher complexity proteomic profiling methods are established, they should be able to be utilized within this same conceptual framework. Less clear is whether or not specific new insights into signal transduction in leukemia were garnered by this study. Similarly, the sparseness of the data makes it difficult to form a true network understanding of signaling in these cells.

Nevertheless, the study does raise the provocative notion that a functional taxonomy of cancer—that is, a taxonomy built on functional response (however measured) to a diverse set of cellular perturbations—could be highly informative. Of course there are at present numerous technical limitations to the widespread application of this approach to solid tumors, but the principle is indeed established, and the study will hopefully prompt others to use the tools of genomics and high dimensionality data analysis and bring them to bear on studying the functional consequences of perturbation of cancer cells. Such efforts will at last put the *functional* in functional genomics.

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SRCircumventing imatinib resistance

The ABL inhibitor imatinib is a highly effective therapy for patients with chronic myeloid leukemia. Relapses after an initial response have been observed in some patients, and mutations of the *BCR-ABL* gene are the most common mechanism driving these relapses. Alternative ABL inhibitors have been identified that inhibit most of the common BCR-ABL mutations, and one has entered clinical trials. The structural basis for these results has yielded significant insights into the mechanism of action of these compounds, mechanisms of resistance, and their ability to inhibit the BCR-ABL mutants. These studies demonstrate the importance and impact of conducting scientific studies as part of clinical trials.

Imatinib, a selective inhibitor of the ABL tyrosine kinase, is a highly effective treatment for chronic myeloid leukemia (CML), a disease driven by the activated BCR-ABL tyrosine kinase. Relapses after an initial response have occurred in a small percentage of chronic phase patients, but are quite common in patients with advanced disease. In the majority of cases, resistance is caused by reactivation of BCR-ABL kinase activity, indicating that resistance could be overcome if inhibition of BCR-ABL was restored. After the original report of a threonine to isoleucine substitution at amino acid 315 (T315I) (Gorre et al., 2001), it has become clear that mutations in the kinase domain of BCR-ABL are the predominant mechanism underlying acquired drug resistance, although some patients have amplification of BCR-ABL (Hochhaus et al., 2002; Shah et al., 2002). Mutations have now been observed in at least 17 different amino acids scattered throughout the ABL kinase domain and render the kinase variably less sensitive to imatinib (Shah et al., 2002; Corbin et al., 2003).

Once it became clear that resistance to imatinib was frequently due to mutations of BCR-ABL, alternative inhibitors that could inhibit these ABL mutants were sought. The first compound identified with this capability, PD180970, a pyridopyrimidine derivative, had originally been developed as a SRC kinase inhibitor, but was subsequently shown to inhibit wild-type ABL at nanomolar concentrations (Dorsey et al., 2000). Based on structural data discussed later, we reasoned that SRC/ABL inhibitors would likely inhibit the kinase domain mutants detected in patients and showed, with the notable exception of T315I, that PD180970 inhibited all imatinib-resistant BCR-ABL kinase domain mutants tested in vitro (La Rosee et al., 2002). Although the unfavorable pharmacokinetic profile of the pyridopyrimidine derivatives precluded their clinical development, these studies provided proof of principle for the

concept that imatinib resistance due to kinase domain mutations could be overcome with alternative ABL inhibitors. A number of other SRC/ABL inhibitors, including other pyridopyrimidines and AP23464, a trisubstituted purine, have been tested in vitro and consistently demonstrate that imatinib-resistant BCR-ABL kinase domain mutations, with the exception of T315I, are inhibited by these compounds (Huron et al., 2003; O'Hare et al., 2004).

A recent report in *Science* extends these observations on a similar SRC/ABL inhibitor to in vivo studies. In the study by Shah et al., orally administered BMS-354825 was shown to block BCR-ABL kinase activity in a murine leukemia model (Shah et al., 2004). Survival of mice injected with cells expressing wild-type or M351T mutant BCR-ABL was prolonged, while the drug was ineffective against tumors expressing the T315I mutant of BCR-ABL. These results raise hopes that BMS-354825 may be effective in patients with sensitive BCR-ABL mutants and provide an effective treatment option for many patients with imatinib resistance. In addition, the greater potency of the compound may induce responses in patients with overexpression of BCR-ABL. As BMS-354825 is currently in Phase I clinical trials, results from these studies should be available soon.

The crystal structure of ABL with imatinib and a pyridopyrimidine has provided crucial insights into the mechanism of action of imatinib and alternative ABL kinase inhibitors. Kinase domains exhibit a canonical bi-lobar structure, with the catalytic site lining the groove between the N- and C-terminal lobes (Figure 1). Kinase activity is regulated by the position of the activation loop, a flexible structure that assumes distinct conformations in the inactive and active states. Unexpectedly, imatinib binds to the ABL kinase domain in its unique inactive conformation, with the activation loop in the closed position (Schindler et al., 2000). Given that the activation loops of active kinases assume very similar conformations, while their inactive conformations are distinct, this explained the ability of imatinib to inhibit ABL, but not SRC. These two kinases have a high degree of amino acid homology in their kinase domains, but adopt unique conformations in their inactive states. In contrast to imatinib, the pyridopyrimidine derivative PD173955 was found to bind to ABL independently of the conformation of the activation loop, indicating that binding was not influenced by the activation state of the kinase (Nagar et al., 2002). Another significant difference between the two inhibitors was their mode of binding to the P loop, the site that normally accommodates the phosphate groups of ATP. Whereas the P loop undergoes extensive conformational changes on imatinib binding, there are only minimal rearrangements in the complex with PD173955 (Figures 1 and 2). Since the active conformations of kinases are much more similar than their inactive conformations and the structural requirements for binding compounds such as PD173955 are much less stringent than imatinib, it is likely that PD173955 and related compounds such as BMS-354825 will be less specific than imatinib. This raises the possibility that these compounds could have more long-term side effects, including immunosuppression due to SRC inhibition.

These crystal structures have also

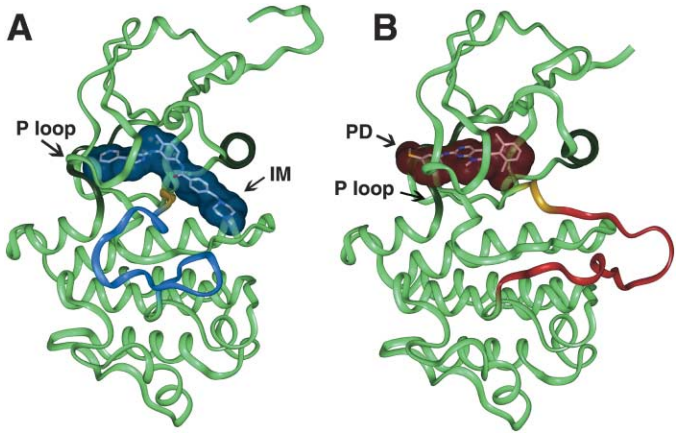

Figure 1. Imatinib (IM) and PD173955 (PD) in complex with the ABL kinase domain

A: Imatinib occupies most of the interlobar groove of the kinase domain, capturing the kinase in a unique inactive conformation. The activation loop (blue) is in the closed position, preventing substrate binding.

B: PD173955 occupies much less surface area. Most importantly, the kinase is in an active state with the activation loop (red) in an open conformation. Note that imatinib would encounter a significant steric clash with the activation loop in the active conformation. Modified from Nagar et al. (2002) with permission.

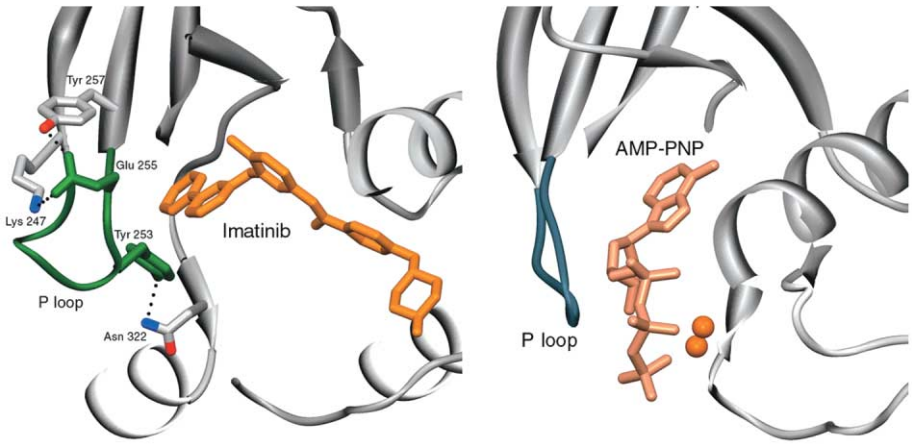

Figure 2. Structural changes in the P loop induced by imatinib binding

The P loop of the active insulin receptor kinase (right) bound to a nucleotide is shown. Note the nearly vertical position of the P loop in this structure. On the left, the massive distortions induced in the P loop (green) to accommodate imatinib binding are shown. Modified from Shah et al. (2002) with permission.

provided significant insights into the mechanism of resistance of the various ABL mutants to imatinib and why they are sensitive to the SRC/ABL inhibitors. Imatinib contacts more than 20 amino acids in the ABL kinase domain, most of which are not contact sites for PD173955. Substitutions of any of these residues can lead to a steric clash with imatinib, but not with PD173955. Mutations within the P loop may impair the ability of the P loop to undergo the extensive conformation changes that are required for imatinib binding. These mutations would be much less likely to block the minor conformational adjustment necessary for binding of pyridopyrimidines. Other mutations probably result in a kinase in which the open or active conformation of the activation loop is favored. As shown in Figure 1B, PD173955 can bind to this conformation of the kinase, but imatinib encounters a significant steric clash with the activation loop in this open conformation. This mechanism is likely responsible for the resistance of activation loop mutants and of mutations that affect sites with a role for the auto-inhibition of ABL. This leaves the T315I mutant, which is resistant to all inhibitors reported thus far. T315 makes a hydrogen bond with imatinib, and there is a van der Waals interaction of this residue with PD173955. Thus, resistance could be due to loss of this interaction. It is also possible that any bulky substitution at position 315 prevents drug binding due to steric hindrance. It should be emphasized that the crystal structure of the ABL kinase domain in complex with alternative ABL inhibitors other than PD173955 has not yet been reported. However, the fact that their activity profiles against the various mutants are comparable argues that they have a similar mode of binding.

An interesting prospect is that combinations of imatinib with BMS-354825 may delay or prevent the emergence of resistant clones, analogous to targeted therapy of HIV infection. Most CML patients on imatinib, including those treated in early chronic phase, harbor residual leukemia. The precise mechanism underlying the persistence of residual disease has not been defined, but it is conceivable that a more potent ABL kinase inhibitor may be capable of eradicating these residual leukemic cells. Alternatively, low levels of mutant clones could contribute to the disease persistence, and alternative ABL inhibitors could also allow the eradication of these leukemic cells. The success of this approach will ultimately depend on the prevalence of T315I mutant clones and their contribution to disease persistence. Many patients with imatinib resistance harbor multiple different mutations (Shah et al., 2002), and the frequency of "occult" T315I mutant clones could be higher than expected. Thus, even under the selective pressure of an inhibitor combination, the resistant T315I mutant may emerge as the default mutant. It is also possible that hitherto unknown resistant mutants will be selected.

Regardless, all of this is good news for patients with CML. First there was the identification and characterization of imatinib. Then, there was the identification of the mechanism of resistance and the discovery of compounds capable of circumventing resistance. Now there is a clinically viable compound that has entered clinical trials for patients with imatinib resistance, and we eagerly await the results of these trials.

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