**Technical Comments**

**Roots of Clinical Resistance to STI-571 Cancer Therapy**

STI-571, a new Abl tyrosine kinase inhibitor recently approved by the Food and Drug Administration, is highly effective in treating the early stages of chronic myeloid leukemia (CML), but remissions induced in advanced phases tend to be relatively short-lived, an observation that suggests the development of de novo resistance to the drug (1, 2). Gorre et al. (3) showed that “acquired” resistance to STI-571 is usually associated with reactivation of the tyrosine kinase activity of the BCR-ABL oncoprotein; this was due to amplification of the gene in three patients and to a point mutation in the kinase domain of BCR-ABL in six cases. Although the former mechanism had been previously identified in CML cell lines (4–6), Abl mutations affecting the binding of STI-571 are novel. It is plausible that specific amino acid substitutions should lead to drug resistance, because crystallographic studies have shown that some amino acid residues within the ATP pocket of the kinase domain are essential for STI-571 binding. We find it surprising, however, that the same nucleotide change (ACT \→\ ATT) was found in six of nine patients who developed STI-571 resistance.

Like Gorre et al., we have been investigating the mechanisms of acquired resistance in clinical material from CML patients and acute leukemia patients positive for the Philadelphia (Ph) chromosome translocation who are receiving treatment with STI-571. We sequenced the BCR-ABL kinase domain from cells obtained from 12 patients who relapsed while receiving STI-571 and identified a functional point mutation in the kinase domain in only one case. This was a G \→\ A change that results in a Glu \→\ Lys substitution at position 255 of Abl. In none of the 12 cases did we find the Thr315Ile mutation identified by Gorre et al. Hochhaus et al. report similar findings in an accompanying comment, below; their study identified a different point mutation in the ATP binding site of BCR-ABL tyrosine kinase in one out of 32 patients studied, and none of their cases had the Thr315Ile substitution.

The big difference in frequency of the Thr315Ile mutation between the North American study, which found the mutation in six of nine patients, and the two European studies, which found it in none of the 44 patients examined, is intriguing. Could it be due to differing ethnic or genetic backgrounds in the patient populations? Or were the individuals studied by Gorre et al. (3) heavily pretreated with high-dose radiotherapy or chemotherapy that increased the incidence of specific mutations? In either case, it would still be difficult to explain the finding of one unique nucleotide change in all the mutated cases without coexistence of at least some other mutations. The apparent “cluster of identical mutations” could be an artifact due to PCR contamination, but this is unlikely, because the sequence was confirmed in genomic DNA in some cases. Alternatively, one could argue that the negative results in the two European studies were due to low sensitivity of detection—also unlikely, because other, less frequent point mutations were readily identified in both patient cohorts. Thus, we believe that it is probably premature to assume that the main cause of resistance to STI-571 in CML patients is one specific mutation in the BCR-ABL kinase domain.

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Despite striking hematologic and cytogenetic responses in the majority of CML patients treated with STI-571, primary or acquired resistance has been observed in a significant proportion of patients in advanced-phase disease (1). Gorre et al. (2) reported that resistance to STI-571 is associated with reactivation of BCR-ABL signal transduction: Of nine cases studied, six had acquired an identical point mutation within BCR-ABL that resulted in a Thr315Ile substitution within the ATP binding pocket.

To determine whether acquired mutations have a role in STI-571 resistance, we investigated a larger group of patients (n = 32) who were either refractory to treatment or who relapsed while being treated. The median duration of therapy was 95 days; prior to STI-571 treatment, two patients were in chronic phase, nine in accelerated phase, 20 in myeloid, and one in the lymphoid blast crisis of the disease. We sequenced reverse transcriptase–polymerase chain reaction (RT-PCR) products specific for the BCR-ABL tyrosine kinase domain (3), and, in contrast to the findings of Gorre et al., detected no mutation that would affect amino acid 315 in any individual.

An acquired A \→\ T point mutation at position 58802 (4)—however—which is predicted to result in a Glu255Val substitution—was detected in one patient. Restriction analysis of cDNA and genomic DNA (5) was used to confirm the presence of the mutation and to track it during the course of treatment. Only wild-type Abl sequence was present before the STI-571 therapy. The patient was treated with STI-571 in late chronic phase and went into complete hematologic remission, but progressed to blast crisis after five months. Reactivation of BCR-ABL was confirmed by Crkl immunoblotting (6). The relative proportion of phosphorylated Crkl (reflecting active BCR-ABL) was 49% before STI-571 therapy, 24% at day 27, 28% at day 83, and 77% at the time of clinical resistance at day 166.

The biological significance of the Glu255Val change was determined by an Abl autophosphorylation assay. Wild-type Abl showed a median inhibitory concentration (IC50) of 0.025 \mu\ M; the mutation led to a virtual insensitivity to STI-571, with an IC50 of >5 \mu\ M. Barthe et al., in an accompanying comment, above, found a similar mutation (G5801A) resulting in a Glu255lys exchange in one of 12 cases investigated; no cases with Thr315Ile substitution were seen. Thus, new point mutations have been observed in two studies of STI-571–resistant patients, and the recurrent mutation described by Gorre et al. was not found in any individual. The discrepancy between these studies is striking, and the reasons for the discrepancy are not clear. To develop effective strategies that might be used to treat STI-571–resistant patients, determining whether the differences between the studies are attributable to biological or technical factors will be essential. Clearly, mutations in the Abl kinase domain that
render the kinase insensitive to STI-571 can occur, but the frequency of these mutations may be less than reported in (2)—and the data reported here suggests that, to evaluate for kinase domain mutations, sequencing needs to encompass the entire kinase domain, not just a single amino acid.

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References and Notes
2. M. E. Gorre et al., Science 293, 876 (2001); published online 21 June 2001 (10.1126/science.1062538).
5. A dosage of 900 mg of clindamycin was administered to the eight patients intravenously over a period of 20 minutes. In one patient of the eight studied, the results for whom we consider representative, STI-571 plasma concentrations declined compared to pre-clindamycin levels (considered as 100%) to 21.2% 5 min after treatment and 21.4% 10 min after treatment.

Response: Our recent study examining the mechanism of clinical relapse in patients with advanced stage CML or Philadelphia chromosome-positive acute lymphoid leukemia (Ph+ ALL) reported that 11 of 11 patients treated with the kinase inhibitor STI-571 had reactivation of BCR-ABL signal transduction at the time of relapse (1). We are pleased to see that the authors of all three comments are in accord with this primary conclusion of our manuscript, which reinforces the crucial role for BCR-ABL even at the most advanced stages of this disease.

We also provided evidence that resistance in this subgroup of patients is due to cell-intrinsic changes. Leukemia cells obtained at relapse showed reduced STI-571 sensitivity compared with pretreatment samples and had acquired new molecular changes such as BCR-ABL gene amplification or mutation. Gambacorti-Passerini et al. make the point that host or “organismic” factors may still play a role in clinical STI-571 drug resistance by showing that drugs such as clindamycin can decrease the plasma level of STI-571, presumably by increasing the level of active STI-571 through displacement from AGP. Although we cannot exclude a role for “organismic” factors, we do not agree that the data of Gambacorti-Passerini et al. provide strong support for this model. One prediction of the “organismic” hypothesis is that plasma STI-571 concentrations and AGP levels in patients at relapse should differ from levels seen at initiation of therapy. We are unaware of such data for AGP; however, we failed to see changes in plasma STI-571 concentrations in relapsing patients studied in the phase I clinical trial (2). In addition to correlating relapse with changes in free and AGP-bound STI-571, validation of this hypothesis requires that interventions that increase free...
STI-571 concentration induce clinical remissions in relapsing patients or prevent the emergence of resistance in responding patients. Recent clinical data indicate that dose escalation of STI-571 in relapsing blast crisis patients has not produced remissions (3).

The comments of Barthe et al. and Hochhaus et al. are notable for the failure of these groups to find the T315I BCR-ABL kinase domain mutation in populations of 12 and 32 CML patients, respectively, whereas we detected this mutation in six of 11 patients (4). At least one other group has independently detected the T315I mutation in Ph+ ALL (5); therefore, it is unlikely that the mutation is an artifact or contaminant. In addition, we have found additional examples of the T315I mutation in patients with CML in myeloid blast crisis and in Ph+ ALL patients. We assume, therefore, that the discrepancy between our data and the results of Barthe et al. and Hochhaus et al. may be due to differences in the patient population studied or the methods used to detect the mutation.

Because the comments do not provide specific details for the European studies, we can only address this question by reviewing our own patient population and methodology. The 11 patients described in our study obtained complete hematologic remissions and, in some cases, complete cytogenetic remissions on STI-571, then relapsed within two to six months. This clinical scenario must be distinguished from those of patients who obtain only partial responses to STI-571 or fail to respond at all. In a phase II trial of 260 patients treated with STI-571 in myeloid blast crisis (3), only about 20% of patients fell into the former group. Therefore, the 11 patients described in our study represented a highly select population. This distinction is important, because patients with partial hematologic responses and no cytogenetic response will have a substantial number of mature BCR-ABL expressing hematopoietic cells that persist during treatment and are not representative of the relapsing, drug-resistant subclone. Since the current protocols for mutation detection do not specifically isolate relapsing, drug-resistant cells from other BCR-ABL expressing blood cells, failure to detect a mutation might be explained by an insensitive assay. In contrast, the dominant population of BCR-ABL expressing cells in patients who relapse after a cytogenetic response will, by definition, be representative of the resistant subclone. Indeed, we found the T315I mutation in more than 80% of BCR-ABL expressing cells from three such patients.

With respect to methodology, we cloned our PCR products rather than perform direct sequencing, and we sequenced at least 10 independent clones per patient. All mutations required confirmation by sequencing in both directions. We chose this strategy to maximize our sensitivity of detecting mutations that may be present in a minority of BCR-ABL–expressing cells. In addition, this method provided a rough quantitative estimate of the fraction of BCR-ABL–expressing cells that contained the mutation, so that clonal evolution could be monitored over time. In retrospect, this method allowed us to find the T315I mutation in several patients in whom the resistant clone represented less than 20% of the BCR-ABL–expressing cells.

As stated in (1), our principal goal was to characterize the role of the BCR-ABL signaling pathway in STI-571 resistance through careful study of a limited number of selected patients. Having demonstrated that BCR-ABL kinase domain mutations can occur, we have undertaken further studies to define the range of mutations and the frequency with which they occur in different clinical situations. Sequencing analysis of 18 additional patients with myeloid blast crisis has identified three more cases with the T315I mutation and four cases of an E255K mutation (6); the latter mutation was also found in two patients in the European studies. Although it is too early to make definitive conclusions about mutation frequency, the combined data from (1) and our more recent analysis show detection of the T315I mutation in nine of 29 patients (six of 25 with myeloid blast crisis and three of four with Ph+ ALL or lymphoid blast crisis) and the E255K mutation in four of 29 patients. We agree completely with the recommendations of Barthe et al. and Hochhaus et al. that future analysis of clinical material should focus on sequence analysis of the entire BCR-ABL kinase domain.

As more data are accumulated from analyses of larger numbers of patients, it should be possible to address a number of key questions: Do different mutations segregate with different clinical phenotypes (i.e., lymphoid versus myeloid disease) or with different clinical patterns of STI-571 resistance (refractory disease; delayed relapse versus rapid relapse)? Are kinase domain mutations restricted to patients with advanced stage disease or do they occur in chronic phase patients? Are these mutations a manifestation of clonal diversity and genetic instability associated with disease progression, are they a consequence of prior exposure to chemotherapy, or do they occur only in patients exposed to STI-571? More investigation is required to sort out these issues, and the answers are likely to have implications for other targeted kinase inhibitors currently in clinical development. We are pleased that our initial report of BCR-ABL kinase domain mutations has generated excitement about this question in the scientific community.

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4. As noted in (1), a 579-base pair region corresponding to the ATP binding pocket and the activation loop of the kinase domain of BCR-ABL was sequenced in the nine of 11 patients for whom RNA was available at the time of relapse. Hence, in their comments, Barthe et al. and Hochhaus et al. refer to six of nine patients rather than six of 11.
5. P. Koehler et al., personal communication.

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