Perspectives on the development of a molecularly targeted agent

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STI571 (Gleevec, imatinib mesylate) exemplifies the successful development of a rationally designed, molecularly targeted therapy for the treatment of a specific cancer. This article reviews the identification of Bcr-Abl as a therapeutic target in chronic myelogenous leukemia and the steps in the development of an agent to specifically inactivate this abnormality. Issues related to clinical trials of molecularly targeted agents are discussed, including dose and patient selection, as are possible mechanisms of resistance to STI571. Lastly, the potential use of STI571 in other malignancies and the translation of this paradigm to other malignancies is explored.

The introduction of STI571, an agent targeted against the causative molecular event in chronic myeloid leukemia (CML), has been heralded as a major advance in the treatment of cancer (Chabner, 2001; Von Hoff and Bootman, 2001). STI571 owes much to the rich history of scientific discovery in CML beginning with the description of the Philadelphia chromosome in 1960 (Nowell and Hungerford, 1960). Many of the scientific discoveries in CML were firsts in cancer research, while others paralleled the state of the art in cancer research (Druker, 2001; Mauro et al., 2002; Rowley, 2001). Thus, STI571 has validated nearly four decades of cancer research into the molecular etiology of cancer and has substantiated the concept that a precise understanding of the pathogenesis of a cancer can lead to effective therapies. This article will provide an overview of the development of STI571 along with the lessons learned in the clinical trials of this molecular pathogenetically targeted agent.

Chronic myeloid leukemia

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder. It accounts for 15%–20% of all cases of leukemia with an annual incidence of 1 to 2 cases per 100,000 per year. The median age at diagnosis is between 50 and 60 years of age. Clinically, the disease progresses through distinct phases referred to as chronic, accelerated, and blast. The chronic or stable phase of the disease is characterized by excess numbers of myeloid cells that differentiate normally. Within an average of 4–6 years, the disease transforms through an "accelerated phase" to an invariably fatal acute leukemia, also known as blast crisis. Disease progression is likely due to the accumulation of molecular abnormalities that lead to a progressive loss of the capacity for terminal differentiation of the leukemic clone (Faderl et al., 1999; Sawyers, 1999). The accelerated and blast phases of the disease are highly refractory to therapy. Response rates to standard chemotherapy in myeloid blast crisis are 20% or less and the median survival is 2–3 months (Faderl et al., 1999; Sacchi et al., 1999; Sawyers, 1999).

Bcr-Abl as a therapeutic target in CML

The hallmark of CML is the Philadelphia chromosome. First identified in 1960 as a shortened chromosome in marrows of CML patients, it was subsequently shown to result from a reciprocal translocation between chromosomes 9 and 22 (Nowell and Hungerford, 1960; Rowley, 1973). The molecular consequence of this translocation is the replacement of the first exon of c-*abl* with sequences from the *bcr* gene resulting in a *bcr-abl* fusion gene and mRNA (de Klein et al., 1982; Groffen et al., 1984; Heisterkamp et al., 1983; Shtivelman et al., 1985). The resulting Bcr-Abl fusion protein has enhanced tyrosine kinase activity (Konopka et al., 1984). Bcr-Abl is present in 95% of patients with CML. In a variety of animal models, Bcr-Abl, as the sole oncogenic event, has been conclusively established as a leukemic oncogene (Daley et al., 1990; Heisterkamp et al., 1990; Huettner et al., 2000; Kelliher et al., 1990). Bcr-Abl functions as a constitutively activated tyrosine kinase, and this kinase activity leads to the pathological defects of CML. These include increased proliferation, protection from programmed cell death, and possibly genetic instability that leads to disease progression (Deininger et al., 2000). However, all of the transforming functions of the Bcr-Abl protein are dependent on the tyrosine kinase activity of the Abl portion of this protein (Lugo et al., 1990). Thus, an inhibitor of the Abl protein tyrosine kinase would be predicted to be an effective and selective therapeutic agent for CML.

STI571 as a therapeutic agent for CML

STI571 (formerly CGP 57148, now imatinib mesylate, Gleevec, or Glivec) was synthesized in a kinase inhibitor program at Novartis, with one of the targets being the platelet-derived growth factor receptor (PDGF-R) tyrosine kinase. The most potent molecules generated were inhibitors of the Abl and the PDGF-R-α and β kinases (Buchdunger et al., 2001; Druker and Lydon, 2000). STI571 emerged as the lead compound for clinical development based on its superior in vitro selectivity against CML cells and its drug-like properties, including pharmacokinetic and formulation properties (Druker and Lydon, 2000). Extensive profiling of STI571 has shown that the only other kinase inhibited by STI571 is the Kit tyrosine kinase (Druker and Lydon, 2000).

Preclinical studies showed that STI571 specifically inhibits the proliferation of cells expressing Bcr-Abl in vitro and in vivo (Deininger et al., 1997; Druker et al., 1996; Gambacorti-Passerini et al., 1997). STI571 levels of 1 μM were optimal for cell killing in vitro (Buchdunger et al., 2001). Studies in mice showed that continuous exposure to STI571 was necessary to eradicate the tumors (Deininger et al., 1997; Druker et al., 1996; Gambacorti-Passerini et al., 1997), suggesting this would be important for optimal anti-leukemic effects. Prior to clinical testing, STI571 was shown to have an acceptable animal toxicology profile.

Clinical studies of STI571

In Phase I clinical trials of STI571, 14 patient cohorts were treat-

ed with escalating daily oral doses of STI571, ranging from 25 to 1000 mg (Druker et al., 2001b). In these studies, no dose-limiting toxicities were observed. Once doses of 300 mg or greater were reached, significant clinical benefits were observed. In chronic phase patients who had failed standard therapy with interferon, 53/54 patients achieved a complete hematologic response, typically within 4 weeks of starting therapy. 51/53 patients maintained normal blood counts after one year of therapy (Druker et al., 2001b). In myeloid blast crisis patients, 60% of patients responded to therapy with STI571, but only 18% of patients had responses lasting longer than one year (Druker et al., 2001a).

The Phase I results were confirmed in rapidly accruing Phase II studies (Kantarjian et al., 2001; Sawyers et al., 2001; Talpaz et al., 2001). In 532 chronic phase patients who failed interferon therapy, 95% of patients achieved a complete hematologic response with STI571 at 400 mg per day. 60% of patients had a reduction in the percentage of Philadelphia chromosome positive metaphases to less than 35%, and 41% of patients achieved a complete cytogenetic remission. With a median duration of followup of 18 months, only 9% of patients have relapsed (Kantarjian et al., 2001). In myeloid blast crisis, of 232 patients treated, 52% responded, but only 22% had responses lasting longer than one year (Sawyers et al., 2001).

Toxicity from therapy has been relatively mild. Only 2% of chronic phase patients discontinued therapy due to adverse events (Kantarjian et al., 2001). The most common side effects have been mild and include nausea, periorbital edema, muscle cramps, and myelosuppression (Druker et al., 2001b). The rare serious adverse events have included skin rashes, fluid retention, elevated liver function tests, and prolonged myelosuppression. The incidence of myelosuppression is much greater in CML patients than in solid tumor patients (Blanke et al., 2001). This suggests that myelosuppression is a therapeutic effect in CML patients where hematopoiesis is predominantly contributed by the Bcr-Abl positive clone.

Why was STI571 so successful?

There were several important elements to the success of STI571, but perhaps none as important as the target itself, Bcr-Abl. As noted above, Bcr-Abl is the causative molecular abnormality of CML and early in the disease course may be the sole oncogenic event. All of the transforming functions of Bcr-Abl are dependent on its tyrosine kinase activity; thus an inhibitor of this kinase would be predicted to be an effective therapeutic agent.

An equally as important issue is that the initial clinical trials were limited to patients with Bcr-Abl positive CML. As STI571 is an Abl inhibitor, it would make sense to try this agent in a disease that was known to be critically dependent on Abl kinase activity. CML is ideal as 95% of patients are Bcr-Abl positive and can be easily identified by the presence of the Ph chromosome, by FISH analysis for Bcr-Abl rearrangements, or by RT-PCR for Bcr-Abl transcripts. Thus, patients were selected for the clinical trial based on the presence of an appropriate target for STI571.

Lastly, CML is a disease that exemplifies the concept of multistage tumor progression. Early in the course of the disease, it is likely the Bcr-Abl is the sole molecular abnormality. Over time, the leukemic clone accumulates additional molecular changes as the disease progresses toward blast crisis. As seen in the clinical trials, the response rate and durability of responses is greater in chronic phase patients than patients with more advanced disease. Even in blast crisis patients, Bcr-Abl remains a good target as in the majority of patients, the malignant clone remains at least partially dependent on Bcr-Abl kinase activity for survival (Druker et al., 2001a). However, STI571 alone is clearly insufficient for most blast crisis patients.

In reflecting on the success of STI571, it is clear that combining a critical pathogenetic target that is easily identifiable early in the course of the disease with an agent that targets this abnormality led to the remarkable results observed in the clinical trials of STI571. In this scenario, early stage CML may be more akin to a carcinoma in situ. To recapitulate the success of STI571 in other malignancies, it would be essential to identify early pathogenetic changes. In parallel, it would be necessary to develop extremely reliable techniques to identify patients with early stage disease.

Why have side effects of STI571 been so minimal?

One of the surprising aspects of the clinical trials was how well tolerated this therapy has been. Abl null animals are viable, but have a variety of neurological defects, lymphopenia and typically die shortly after birth (Schwartzberg et al., 1991; Tybulewicz et al., 1991). Animals lacking either the PDGF-R-α or β die in early gestation with a variety of developmental defects, including neural crest abnormalities (α) and renal, cardiovascular, and hematological abnormalities (β) (Soriano, 1994, 1997). Inactivating mutations of Kit results in embryonic lethality due to severe anemia (Russell, 1979). These animals also have deficiencies in melanocytes, germ cells, and mast cells (Dolci et al., 1991). Based on these observations, it would seem likely that significant side effects would be observed with STI571.

The lack of side effects of STI571 might be due to the lack of a critical role for the kinases targeted by STI571 in an adult animal as opposed to their critical role during embryonic development. Alternatively, humans may have redundant gene function compared with mice that minimizes the effect of loss of these kinases. Lastly, it is possible that residual kinase activity may persist during STI571 therapy and this may be sufficient to protect dependent tissues. If this latter explanation is correct, then side effects might become more apparent as more potent kinase inhibitors are generated.

Why do cytogenetic responses occur?

Bcr-Abl functions either by enhancing the proliferation of a hematopoietic progenitor or by protecting progenitors from programmed cell death (Deininger et al., 2000). In the former case, it is possible that STI571 would simply prevent proliferation of the leukemic clone without eliminating it. Even in this scenario, it is possible that cytogenetic responses would be observed. Presumably, with inhibition of Bcr-Abl kinase activity, the leukemic clone would be subjected to normal marrow regulatory influences and would be eliminated at the end of the progenitor cell's natural life span. It is also possible that normal hematopoietic progenitors would regain a proliferative advantage or that progression to blast crisis could be delayed or avoided. If Bcr-Abl protects from apoptotic cell death, it is possible that treatment with a kinase inhibitor could eliminate the leukemic clone.

However, cytogenetic responses are not seen in all patients. The simplest explanation is that some patients have additional molecular abnormalities in a clone of cells that mediates resistance. Another possibility is that quiescent stem cells may be insensitive to STI571 (Graham et al., 2001), suggesting that complete eradication of the malignant cell with single agent STI571 would be unlikely. Alternatively, different stages of

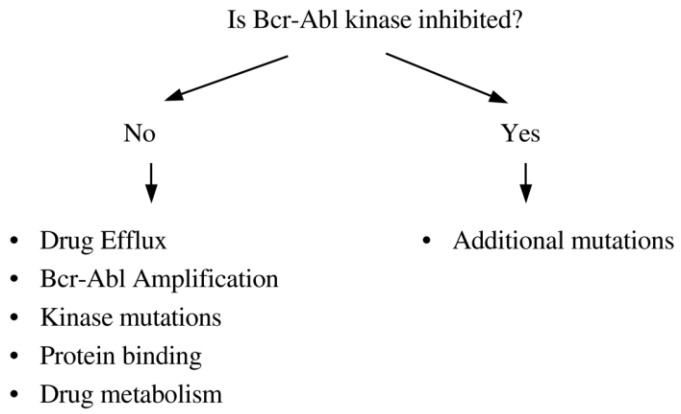

Figure 1. Distinguishing between potential mechanisms of relapse

hematopoietic stem cell development could be involved in the initial pathogenesis of CML. If the stem cells have differential sensitivity to STI571, this may explain the variability in cytogenetic responses.

Dose selection

In the Phase I clinical trials of STI571, a maximally tolerated dose of STI571 was never reached (Druker et al., 2001b). Even among those closely associated with the Phase I studies, there was a lack of consensus about when to discontinue dose escalation. There were some investigators who felt that no cap on the dose should be considered except the maximally tolerated dose, particularly since studies in solid tumors were planned and since penetration of STI571 into solid tumors might require higher doses. Others felt that alternate endpoints, such as optimal therapeutic response and/or pharmacokinetic endpoints, could be used. In evaluating pharmacokinetic endpoints, it was known from preclinical studies that continuous exposure of cells to STI571 doses of 1 μM or higher resulted in maximal cell killing (Druker and Lydon, 2000). In the Phase I clinical trials, a trough level of 1 μM was reached at a dose level of 300 mg, which corresponds to a threshold for significant therapeutic benefits (Peng et al., 2001). Thus, pharmacokinetic parameters could have been used to predict therapeutic responses. Similarly, an analysis of responses in blood counts over time suggested that doses of 400 to 600 mg were on the plateau of a dose-response curve (Peng et al., 2001). Thus, an analysis of optimal therapeutic responses led to a similar conclusion that doses greater than 300 mg should be chosen for Phase II trials. However, the analysis of optimal therapeutic responses may not always be available from early clinical trials, particularly since disease-free survival may be the best parameter to measure.

When using molecularly targeted agents, it would seem more reasonable to consider maximal modulation of the target as the therapeutic endpoint. In the case of CML and a Bcr-Abl inhibitor, the obvious choice would be to assess for maximal inhibition of Bcr-Abl kinase activity. An analysis of Bcr-Abl kinase inhibition, performed by assaying for decreases in phosphorylation of the Bcr-Abl substrate, Crkl, have shown that a plateau in inhibition is seen above 250 mg (Druker et al., 2001b). Additional experiments are being conducted to determine the percentage of Bcr-Abl kinase activity that is being inhibited at these dose levels. Once again, there are advantages in CML in that the tumor cells are easily accessible and that the kinase itself or its substrates could be monitored for inhibition. These types of assays will clearly be more problematic for solid tumors but will likely be necessary to determine the penetration of agent into the tumor. In the absence of specific assays, information about intracellular drug levels in tumor samples would also be a useful surrogate. This type of data, regarding maximal kinase inhibition, could be particularly useful in explaining response variability, determining mechanisms of relapse, and individualizing patient dosing.

Mechanisms of relapse/resistance to STI571

Resistance to STI571 includes de novo resistance and relapse after an initial response. One of the most useful categorizations of relapse/resistance mechanisms has been separation of patients with persistent inhibition of the Bcr-Abl kinase and those with reactivation of the Bcr-Abl kinase (Figure 1). Patients with persistent inhibition of the Bcr-Abl kinase would be predicted to have additional molecular abnormalities besides Bcr-Abl driving the growth and survival of the malignant clone. In contrast, patients with persistent Bcr-Abl kinase activity or reactivation of the kinase would be postulated to have resistance mechanisms that either prevent STI571 from reaching the target or render the target insensitive to Bcr-Abl. In the former category are mechanisms such as drug efflux or protein binding of STI571. In the latter category would be mutations of the Bcr-Abl kinase that render Bcr-Abl insensitive to STI571 and amplification of the Bcr-Abl protein.

In patients who relapse after an initial response to STI571, the majority of patients have reactivation of the Bcr-Abl kinase (Gorre et al., 2001). No changes in drugs levels have been observed, and leukemic cells from these patients have decreased cellular sensitivity to STI571 (Gorre et al., 2001). This suggests that resistance is due to intrinsic cellular properties rather than protein binding of drug or drug metabolism. Interestingly, half of these patients have developed point mutations in the Abl kinase that render the kinase variably less sensitive to STI571 (Branford et al., 2001; Hochhaus et al., 2001; Shah et al., 2001). Two of the point mutations are at sites predicted to be contact sites between STI571 and the Abl kinase based on the crystal structure (Gorre et al., 2001; Barthe et al., 2001; Branford et al., 2001; Hochhaus et al., 2001; Schindler et al., 2000) (Figure 2). Some are at residues adjacent to contact points, while others are in the kinase activation loop (Barthe et al., 2001; Branford et al., 2001; Hochhaus et al., 2001; Shah et al., 2001). Lastly, approximately one-third of patients who relapse after an initial response have Bcr-Abl amplification (Hochhaus et al., 2001). In these patients, Bcr-Abl remains a good target for therapy, and inhibitors with activity against the Abl mutations or more potent Abl inhibitors will be required. Bcr-Abl mutation and amplification have not been commonly seen in patients with de novo STI571 resistance, and ongoing studies are aimed at identifying mechanisms of resistance in these patients.

Optimizing therapy with STI571

One of the tasks for the future will be to predict response patterns of patients. This will include predictions based on individual genetic factors that might influence parameters, such as drug metabolism as well as the genetic makeup of the tumor. Current techniques to predict responses use pretreatment clinical features that are likely surrogate markers for specific genet-

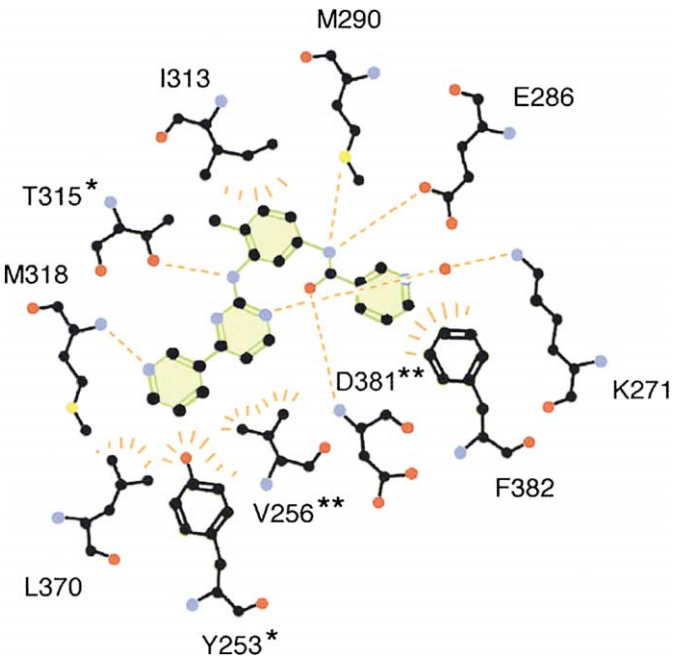

Figure 2. Crystal structure of Abl bound to an STI571-related compound

Dashed lines indicate hydrogen bonding. Curved lines indicate van der Waals interactions. Residues marked with an asterisk are contact points where mutations have been identified in patients with clinical resistance to STI571. Double asterisks are at contact points where mutation of an adjacent residues has been associated with resistance. Reprinted with permission from Schindler et al. (2000).

ic alterations in tumors (Goldman and Druker, 2001). Microarray techniques that survey the gene expression pattern of tumors have already been demonstrated to distinguish subgroups with different clinical outcomes from a larger group of apparently clinically homogenous patients (Alizadeh et al., 2000; Shipp et al., 2000). Thus, the ideal situation would be to predict specific response patterns of patients prior to treatment and treat patients with the minimally toxic, but effective, therapy. In the meantime, STI571 will be combined with standard anti-leukemic agents to increase cytogenetic and molecular response rates in chronic phase patients and to increase durability of responses in advanced phase patients. In the future, additional rational targets for therapy will be identified from studies of molecular mechanisms of resistance.

Translating the success of STI571 to other malignancies

STI571 specifically inhibits the Abl, PDGF-R, and c-kit tyrosine kinases, but no other kinase tested. Initial clinical trials targeted patients with CML where Bcr-Abl tyrosine kinase activity was known to be a pathogenetic event. Shortly thereafter, clinical trials were initiated in GIST patients, where activating mutations of c-kit are a pathogenetic event (Heinrich et al., 2002). Again, remarkable clinical activity was seen in this previously chemotherapy refractory tumor (Blanke et al., 2001; van Oosterom et al., 2001). One of the most interesting findings in the GIST clinical trials is that the response rate to STI571 is significantly higher in patients with mutated/activated c-kit as opposed to patients who express the wild-type, nonmutated c-kit (Blanke et al., 2001). These data demonstrate a critically important concept, that expression does not necessarily equate with pathogenesis.

Kit is expressed in a variety of tumors, including acute myeloid leukemia, small cell lung carcinoma, germ cell tumors, melanoma, myeloma, and neuroblastoma (Heinrich et al., 2002). Whether c-kit contributes to the pathogenesis of these diseases is not clear. It is possible that c-kit tyrosine kinase activity is required for survival of these tumors; however, clinical trials in these disorders will be required to determine the activity of STI571 in these diseases. The Tel-PDGF-R fusion, seen as a consequence of the (5;12) translocation in a subset of chronic myelomonocytic leukemia patients, is a well-validated pathogenetic target, and STI571 has shown remarkable activity in patients with this disease (Apperley et al., 2001; Carroll et al., 1997; Golub et al., 1994). Glioblastomas have a reasonably well-described autocrine activation of the PDGF-R; however, the complexity of these tumors may obscure clinical activity. Despite the presence of the PDGF-R on many other common tumors, the precise contribution of this receptor to the growth and survival of these tumors is not clear. Again, clinical trials of STI571 have been initiated and may define the contribution of this pathway. In the absence of clear validation of the PDGF-R or c-kit, predictions regarding activity in these tumors would be premature. Thus, despite the fact that a molecular targeted agent is being used, we still are left resorting to empiricism to define clinical activity.

Concluding thoughts

As much cause as there is for celebration of the success of STI571, it is worth remembering that the success of STI571 was based in large part on the fact that it targeted the most well validated target in all of oncology. To recapitulate the success of STI571 in other malignancies, it would be essential to identify early pathogenetic changes in all cancers and to develop reliable means to identify patients with these abnormalities. But how many pathogenetic events will characterize each cancer? Will there be few or will there be many? It is also worth remembering that drugs that target pathways that are not pathogenetic events may also be good targets. The best examples are tamoxifen in breast cancer and the anti-androgenic agents in prostate cancer. Mutations or constitutive activation of the estrogen or androgen receptors are not known to be pathogenetic events in breast or prostate cancer, yet the activity of these receptors is critical to the survival of these tumors. Thus, targeting these receptors has been an extremely useful therapeutic approach in these malignancies. This demonstrates that any pathway that is critical to the development, growth, or survival of a cancer could be a useful therapeutic target. Our task is to define these appropriate targets in each cancer and to develop agents that reverse the abnormal function of these proteins. As easy as STI571 made it look, there is still much work to be done.

Acknowledgments

B.J.D. is funded by grants from the NCI, a Specialized Center of Research Award from The Leukemia and Lymphoma Society, a Clinical Scientist Award from the Burroughs Wellcome Fund, and the T. J. Martell Foundation. B.J.D. has served as a consultant to Novartis Pharmaceuticals.

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