Most conventional anticancer agents are nonselective cellular poisons with broad activities against most dividing cells, regardless of whether they are malignant or not; any preferential effects of these agents on cancer cells usually reflect their relatively high rates of cell division. The widely acclaimed success of imatinib mesylate (also known as STI571 or Gleevec, and herein referred to as imatinib) in chronic myeloid leukemia (CML) highlights the enormous possibilities that the development of drugs directed against cancer-specific targets has for improving cancer treatment. Targeted anticancer agents have the potential to favorably influence patient survival by decreasing toxicity and improving disease control. However, recent laboratory and clinical data raise questions about whether new anticancer agents will effectively target relevant subsets of cancer cells. These issues have implications for interpreting results of clinical studies with targeted therapies as well as for the future development of new anticancer treatments.

IMATINIB IN CHRONIC MYELOID LEUKEMIA: A MODEL OF TARGETED THERAPY

The cytogenetic hallmark of CML, the Philadelphia chromosome, has been an obvious candidate for selective targeting. The Philadelphia chromosome abnormality in CML is characterized by a reciprocal translocation between chromosomes 9 and 22, resulting in the transfer of c-ABL gene sequences from chromosome 9 to a site adjacent to the BCR gene sequences on chromosome 22. This translocation produces a 210-kD Bcr-Abl fusion protein (p210Bcr–Abl) that is a constitutively active tyrosine kinase (1). p210Bcr–Abl is not only specific for CML, but the genetic translocation also appears to be the initiating oncogenic event: Mouse bone marrow transduced with a retrovirus encoding p210Bcr–Abl produces disease when transplanted into mice (2). Thus, selective targeting of BCR-ABL should be the consummate therapeutic strategy for CML.

Imatinib was developed as part of a program to identify drugs that block the unregulated activities of the protein kinases expressed in many cancers. Imatinib is a potent and relatively selective inhibitor of the Abl tyrosine kinases, including Bcr-Abl (3). Moreover, imatinib has demonstrated striking selective activity against CML cell lines and clinical progenitors in vitro, inhibiting more than 90% of CML progenitor cell growth at concentrations (1–10 μM) that have little activity against normal hematopoietic progenitors. The clinical activity of imatinib has mirrored its in vitro activity. Many CML patients who are interferon resistant enter complete cytogenetic remissions with imatinib (4), and even those with accelerating disease can achieve excellent responses after imatinib therapy (5). Results from a large, multicenter, randomized study comparing imatinib to interferon plus cytarabine indicate that the most dramatic responses to imatinib are in newly diagnosed CML patients (6). With a median follow-up of 19 months, 76% of patients who received imatinib had a complete cytogenetic response, compared with 14% of patients who received interferon plus cytarabine. Imatinib was also tolerated better than interferon plus cytarabine; however, there was no survival difference between the two study arms (6).

In May 2001, imatinib was initially approved by the Food and Drug Administration as a second-line therapy for CML; the interim results of the multicenter randomized trial (6) led to its recent approval as a first-line therapy for CML. In addition, the new National Comprehensive Cancer Network guidelines for the treatment of CML no longer recommend interferon as the standard of care; only imatinib is recommended as a first-line therapy for patients not undergoing allogeneic transplantation (7). This recommendation was made despite the short follow-up with imatinib relative to the natural history of CML (6) and the established track record of interferon (8). Not only has interferon been proven to prolong the survival of CML patients, but the 10%–20% of patients who achieve a complete cytogenetic remission with interferon have a median survival greater than 10 years, and some of these patients may actually be cured (8). The prevailing wisdom is that the high early-response rates with imatinib will ultimately translate into improved survivals. However, a recent report (9) has suggested that most of the patients who achieved the best responses with imatinib (polymerase chain reaction negativity for the BCR-ABL fusion transcript) may now be showing evidence of disease progression; although the long-term clinical significance of this finding is currently unclear, it raises some concerns about the durability of responses to imatinib.

RESISTANCE TO IMATINIB

Recent data are beginning to shed light on mechanisms responsible for clinical resistance to imatinib. For example, BCR-ABL gene amplification or mutations may weaken the antitumor effects of imatinib (10). In addition, secondary genetic mutations capable of driving BCR-ABL–independent leukemic growth may also be present, even at initial diagnosis (11). However, these genetic mechanisms of resistance may be responsible for only a fraction of the failures of imatinib therapy. CML arises at the level of hematopoietic stem cells, and like their normal counterparts, CML stem cells undergo orderly differentiation. Differentiated cells constitute the bulk of the leukemic cell mass.

Affiliation of authors: Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD.

Correspondence to: Richard J. Jones, MD, Bunting-Blaustein Cancer Research Building, 1650 Orleans St., Rm. 207, Baltimore, MD 21231 (e-mail: rjones@jhmi.edu).

DOI: 10.1093/jnci/djh095

Journal of the National Cancer Institute, Vol. 96, No. 8, © Oxford University Press 2004, all rights reserved.
in CML, whereas the stem cells responsible for disease maintenance are rare (12). Several investigators have now provided evidence that imatinib may have differential effects on CML cells depending on their state of differentiation; whereas imatinib is highly toxic to differentiated CML progenitors, CML stem cells may be relatively or even completely resistant to the drug (13,14). The mechanisms involved in CML stem cell resistance to imatinib are unknown. However, CML stem cells may share biologic properties with their normal counterparts that would make them inherently poor targets for imatinib. For example, both the quiescence of hematopoietic stem cells and their high expression of the multidrug resistance-I gene (15), which encodes an efflux pump protein capable of transporting imatinib out of cells (16), may limit the cellular uptake of imatinib. Moreover, although BCR-ABL provides CML stem cells a growth and survival advantage over their normal counterparts, it is not required for their preservation (12). On the basis of the longevity (possibly greater than 10 years) of their normal counterparts, CML stem cells may survive for years even if BCR-ABL expression is completely inhibited (17); eventually, because of intrinsic genomic instability, CML stem cells and their progeny may develop absolute resistance to imatinib.

CANCER STEM CELLS AND TARGETED THERAPY

The rapid responses induced by imatinib (6) are likely to be a consequence of its impressive activity against mature CML progenitors. Moreover, recent data indicating that these early responses may not be durable (9) could be explained if the rare CML stem cells are resistant to imatinib (13,14). This pattern of clinical activity would be analogous to cutting a dandelion (or other weed) off at ground level—it may appear to produce the desired effect, but only elimination of the root will actually prevent the weed from regrowing. In contrast, the primary activity of interferon may be confined to CML stem cells (18), which may explain the slow, but often durable, responses seen in interferon-treated patients (8). Cytogenetic response (i.e., a substantial reduction in the number of blood cells that harbor the Philadelphia chromosome) has been a reliable surrogate marker for survival in CML patients treated with interferon (8). However, because interferon and imatinib may be targeting different CML cell populations, caution should be exercised in broadening the correlation between cytogenetic response and survival to imatinib-treated patients until data from a longer follow-up are available. Whereas clinical responses to interferon may be actually assaying CML stem cells, early responses to imatinib may merely reflect the fate of differentiated CML cells. Imatinib could even produce complete remissions with undetectable BCR-ABL expression by polymerase chain reaction without affecting CML stem cells that represent less than 0.1% of the CML cell population (12).

CML was the first recognized, and remains the best studied, example of a stem cell malignancy. However, cancer stem cells that are biologically distinct from the differentiated cells that characterize the disease have been demonstrated in acute myeloid leukemia (AML) (19), acute lymphocytic leukemia (ALL) (20), myelodysplastic syndrome (21), breast cancer (22), and multiple myeloma (23). It is possible that most malignancies arise from a rare population of cancer stem cells (24), which may have profound implications for the development of targeted anticancer therapies in general. For example, gemtuzumab ozogamicin (Mylotarg), an anti-CD33 monoclonal antibody conjugated to the cytotoxic agent calicheamicin, has been approved for patients with relapsed AML and is currently being studied in newly diagnosed AML patients. Although most AML cells express the myeloid antigen CD33, the leukemic stem cells in most cases of AML phenotypically resemble immature hematopoietic stem cells (19) and do not express antigens that are specific for more differentiated blood cells, including CD33 (25,26). Similarly, monoclonal antibody conjugates directed against the B cell antigen CD19 expressed by most ALL cells are being studied in ALL patients (27,28). Yet it appears that many cases of ALL also originate from a hematopoietic stem cell that does not express CD19 (20). Therapies that target mature cancer cells may produce clinical improvements and dramatic responses, but they are unlikely to produce long-term remissions if the rare cancer stem cells responsible for maintaining the disease are also not targeted.

It is also possible that therapy directed against targets uniquely expressed by cancer stem cells could be prematurely abandoned if clinical activity is judged solely by standard response criteria. For example, rituximab, a monoclonal antibody against the B cell antigen CD20, has excellent activity in B cell lymphomas and may contribute to curing some patients with these diseases (29). However, its activity in multiple myeloma has been disappointing (30), despite emerging evidence that this disease arises from CD20-positive postgerminal center B cells. These rare myeloma stem cells differentiate into the malignant plasma cells that characterize the disease but that usually do not express CD20 (23,31). The parameters typically used to measure clinical response in myeloma (i.e., monoclonal immunoglobulin levels and the percentage of plasma cells in the bone marrow) primarily measure the effect of treatment on the terminally differentiated plasma cells. However, rituximab’s activity would primarily be against myeloma stem cells, analogous to interferon’s possible activity in CML (18), and the long survival of the malignant plasma cells could obscure such activity. It is possible that a longer duration of rituximab treatment may ultimately have demonstrated clinical responses according to standard criteria by inhibiting new myeloma cell production for a sufficient period of time to allow myeloma plasma cells to undergo spontaneous apoptosis (23).

DRUG DEVELOPMENT FOR CANCER: ARE WE OFF TARGET?

The search for therapies that are specific for cancer cells has focused on differences in gene expression between normal and cancer cells. However, such differences within heterogeneous cancer cell populations must also be considered. Cellular antigens or signaling pathways expressed by cancer cells may not be optimal therapeutic targets if immature cancer stem cells that do not express the targets are also present. Even when the initiating oncogenic event is definitively being targeted, as with imatinib in CML, acquired secondary genetic mutations or inherent properties of cancer stem cells may make the target inaccessible or no longer relevant. It is therefore essential that the development of new anticancer treatments focuses on the pathogenesis and biology of the diseases being treated in addition to the drugs and their specific targets. Moreover, standard clinical response parameters primarily assess the fate of the cancer cells that constitute the bulk of the tumor mass, and thus may potentially...
overestimate (e.g., as with imatinib in CML or gemtuzumab in AML) or underestimate (e.g., as with interferon in CML or rituximab in myeloma) the effect of therapy on a minute population of cancer stem cells. Thus, in the absence of surrogate clinical markers that adequately reflect the biology of the disease, survival should remain a primary endpoint of therapeutic efficacy when studying new treatments—particularly those directed against specific cellular targets.

**REFERENCES**


(9) Mauro MJ, Druker BJ, Kuijl J, Kurilic G, Maziarz RT. Increasing levels of detectable leukemia in imatinib treated CML patients with previously undetectable or very low levels of BCR-ABL. Proc ASCO 2003;22:569.


(23) Blair A, Hogge DE, Sutherland HJ. Most acute myeloid leukemia progenitor cells with long-term proliferative ability in vitro and in vivo have the phenotype CD34(+)/CD71(-)/HLA-DR-. Blood 1998;92:4325–35.


**NOTE**

Manuscript received November 26, 2003; revised February 9, 2004; accepted February 17, 2004.