Imatinib Mesylate Efficiently Achieves Therapeutic Intratumor Concentrations \textit{in Vivo} but Has Limited Activity in a Xenograft Model of Small Cell Lung Cancer


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\textbf{ABSTRACT}

\textbf{Purpose:} Despite recent advances in cancer therapy, long-term survival in small cell lung cancer (SCLC) remains uncommon, underscoring the need for novel therapeutic approaches. Previous studies have identified constitutive expression of the receptor tyrosine kinase, c-Kit, and its ligand, stem cell factor, in a substantial proportion of SCLC specimens. The purpose of this study was to determine whether imatinib mesylate, an inhibitor of c-Kit, could achieve therapeutic concentrations in tumors and in brain (a frequent site of SCLC metastasis) and interfere with SCLC tumor growth \textit{in vivo}.

\textbf{Experimental Design:} Human SCLC tumor cell lines with constitutive c-kit expression and tyrosine phosphorylation (NCI-H209, NCI-H526, and NCI-H1607) were used to establish SCLC tumor xenografts in NCr nude \textit{(nu/nu)}-immunodeficient mice. SCLC tumor-bearing mice were randomly assigned to imatinib or control (water) administered twice a day by oral gavage. Imatinib concentrations in plasma, brain, and tumor were quantitated and correlated with tumor response.

\textbf{Results:} Therapeutic concentrations of imatinib were achieved in plasma and tumor xenografts but not in the brain. Imatinib blocked the constitutive activation of c-kit in SCLC tumor cell lines \textit{in vitro} but had a negligible effect on SCLC xenograft growth \textit{in vivo}.

\textbf{Conclusions:} Orally administered imatinib rapidly reaches therapeutic concentrations in SCLC xenografts, suggesting the feasibility of combining imatinib with other novel or traditional chemotherapeutic agents in SCLC or other solid tumors. The c-Kit signaling pathway does not appear to play a critical role in SCLC proliferation and viability \textit{in vivo}, however, suggesting that imatinib is unlikely to be effective as monotherapy for SCLC.

\textbf{INTRODUCTION}

Small cell lung cancer (SCLC) comprises \textasciitilde 15\% of all lung cancers (1, 2). Although patients often respond favorably to multagent chemotherapy, the durability of response is poor. Less than 5\% of extensive-disease patients survive \textasciitilde 5 years after diagnosis, and only \textasciitilde 20\% of patients with limited stage disease are cured with combined modality therapy (1, 3, 4). Nonetheless, the high proliferative rate of SCLC and its relatively high initial response to chemotherapy suggests an opportunity for the development of effective, novel chemotherapeutic and targeted approaches (5). In the early 1990s, several groups reported the expression of the receptor tyrosine kinase c-Kit (6) in as many as 70\% of SCLC cell lines and tumor specimens (7–10). Coexpression of the c-Kit ligand, stem cell factor (SCF), was also detected in these specimens, suggesting the possibility of a c-Kit/SCF autocrine pathway in the pathogenesis of SCLC. This hypothesis was additionally strengthened when subsequent studies reported that manipulations of the c-Kit/SCF pathway altered SCLC chemotaxis, proliferation, and survival (11–14).

The recent release of the tyrosine kinase inhibitor, imatinib mesylate (Gleevec; Novartis), which is a potent inhibitor of c-Kit (15, 16), provides a convenient means to target the Kit/SCF pathway in SCLC. This strategy is attractive in light of recent findings that suggest c-Kit expression is an independent negative prognostic factor for SCLC (17). Imatinib, also a potent inhibitor of platelet-derived growth factor receptor, Abl (15, 18), and Abl-related gene kinase (19) have considerable activity in Philadelphia chromosome-positive hematological malignancies (20, 21) and gastrointestinal intestinal stromal tumors (22, 23), with relatively modest toxicity. In SCLC cell lines, imatinib has been demonstrated to exert either a cytostatic effect (24) or induce modest apoptosis (25) at concentrations of 0.3–5 \textmu M \textit{in vitro}. However, given the limited treatment options available to patients with refractory or relapsed SCLC, novel therapeutic agents capable of even disease stabilization are of considerable interest.

To determine the activity of imatinib against SCLC \textit{in vivo}, we have established a murine SCLC xenograft model using human SCLC cell lines constitutively expressing c-Kit. Tumor-bearing animals were treated orally with imatinib or placebo, and the therapeutic response was correlated with plasma and intratumoral drug concentrations. Imatinib was well tolerated.
and rapidly reached intratumoral concentrations in the expected range for inhibition of c-Kit tyrosine kinase activity. Unfortunately, imatinib had little activity against SCLC xenografts growth in vivo, suggesting the need for a combination approach in the c-Kit targeted therapy of SCLC.

MATERIALS AND METHODS

Cells and Cell Lines. The SCLC lines NCI-H69, NCI-H82, NCI-H187, NCI-H209, NCI-H378, NCI-H524, NCI-H526, NCI-H889, NCI-H1607, NCI-H2107, NCI-H2171, NCI-H2195, and NCI-H2227 (hereafter referred to without the NCI designation) were provided by the Hamon Center Tumor Cell Repository (Dr. Adi Gazdar) at the University of Texas Southwestern Medical Center. Cells were propagated at 5% CO2 in RPMI 1640 (JRH Bio-Sciences, Lenexa, KS) supplemented with 10% FCS, penicillin/streptomycin, and glutamine (Gemini Bio Products, Woodland, CA). NIH-3T3 cells were grown in DMEM (high glucose) supplemented with 10% bovine calf serum. Mo7e cells, used as a positive control for c-Kit expression, were provided by Dr. Martin Sattler (Dana-Farber Cancer Institute, Boston, MA) and propagated in DMEM supplemented with 20% heat-inactivated FCS, 10 ng/ml granulocyte-macrophage colony stimulation factor (Immune Corporation, Seattle, WA), and 2 mM glutamine. As a control for c-Kit activation, H526 cells were incubated overnight in serum-free medium containing 0.5% BSA and then stimulated the following morning with 50 ng/ml SCF (Peprotech, Rocky Hill, NJ) for 7.5 min. To inhibit the constitutive activation of c-Kit, human SCLC cell lines were treated with 0.1–3 μM imatinib mesylate or control (water) overnight and then harvested for protein lysates as described below.

Western Immunoblot and Immunoprecipitation. Cells were lysed in radioimmunoprecipitation assay buffer [50 mM Tris (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 1 mM EDTA, 1% Triton X-100, and 0.1% SDS] containing 1 mM phenylmethylsulfonyl fluoride, 1% aprotinin (V/v), 25 mM sodium fluoride, 1 mM sodium orthovanadate, 1 μg/ml pepstatin, and 2 mM Pefabloc SC (Roche Diagnostics, Mannheim, Germany). Lysates were normalized by Assay (Bio-Rad protein assay; Bio-Rad Laboratories, Hercules, CA) and resolved by SDS-PAGE. Gels were electrophoretically transferred to a nitrocellulose membrane overnight and analyzed by anti-c-Kit (DAKO Corp., Carpinteria, CA), anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA), or anti-phosphotyrosine (4G10; Upstate Biotechnology, Lake Placid, NY) Western immunoblot using enhanced chemiluminescence (Amersham, Piscataway, NJ). For anti-c-Kit immunoprecipitation of human SCLC cell lines, lysates were prepared from 1–2 × 10^7 cells, normalized by Assay nm, and immunoprecipitated with 2 μg of anti-c-Kit antibody (DAKO) overnight at 4°C.

For preparation of SCLC tumor protein lysates, tumors were removed from mice within 2–4 h after the last imatinib (or control) dose and homogenized in radioimmunoprecipitation assay buffer using a Brinkman Mechanical Homogenizer (model PT 10/35). The homogenate was incubated on a rocker at 4°C for 30 min and then centrifuged at 16,000 × g for 10 min to remove the insoluble debris. Protein lysates from 1–2.2 cm^3 sections of tumor were normalized by Assay nm and immunoprecipitated overnight with 5 μg of c-Kit antibody (DAKO) and analyzed by anti-c-Kit or anti-phosphotyrosine Western immunoblot.

Tumor Cell Injection, Tumor Measurement, and Imatinib Administration. Four- to 6-week-old, immunodeficient NCr Nude (nu/nu) mice were obtained from Taconic Farms (Germantown, NY). Animals were treated according to an established animal protocol approved by the Institutional Animal Care and Use Committee at University of Texas Southwestern Medical Center. To facilitate uniform xenograft establishment, mice were irradiated with 350 cGy 24 h before tumor cell injection. To establish SCLC tumor xenografts, 5–10 × 10^6 SCLC cells in a volume of 200 μl of PBS were introduced into the right flank of NCr nude mice by s.c. injection using a 22-gauge needle. Tumor measurements were acquired using a digital caliper and associated software (MouseLog, developed by Luc Girard and Dwight Randle; University of Texas Southwestern Medical Center, Dallas, TX). Triplicate measurements of tumor length (L), height (H), and width (W) were performed, averaged, and used to calculate a tumor volume (L × W × H) for each time point. Tumor measurements were performed at least 3 days/week.

Tumor doubling calculations were determined by the equation \( V_F = V_I e^{kt} \), where \( V_F \) is final tumor volume, \( V_I \) is initial tumor volume, and \( t \) is the number of days between the \( V_F \) and \( V_I \) measurements. According to this formula, tumor doubling time (in days) equals 0.693 divided by \( k \), where \( k = \ln (V_F/V_I) \). The average tumor doubling time (±SE) was determined for each cohort of imatinib- and placebo-treated mice and analyzed for statistical significance using a two-tailed \( t \) test.

When tumors reached 0.3–0.6 cm^3, mice were started on imatinib or placebo. Imatinib (provided by Dr. Elisabeth Buchdunger, Novartis Pharmaceuticals, Basel, Switzerland) was administered by oral gavage as previously described (26) at a dose of 100 mg/kg twice/day. Mice were generally dosed at 9 a.m. and at 6:30 p.m. each day. Placebo mice were treated with sterile water alone. For the imatinib pharmacokinetic experiments, tumor-bearing, imatinib-treated mice were euthanized and promptly autopsied 2, 4, 8, and 15 h after the last dose at steady state, and parallel plasma, brain, and tumor specimens were obtained and processed as described below. Treatment was halted when tumors reached a volume of 2 cm^3. In the case of tumor ulceration, final tumor measurements were performed, and the mouse was euthanized.

Imatinib Concentration Determination. Concentrations of imatinib and its metabolite, CGP 74588, in plasma, tumor, and brain, were determined with a validated liquid chromatography/mass spectroscopy assay (27). Plasma samples were assayed directly. Tumors and brain samples were homogenized in three parts PBS, and 200 μl of each homogenate assayed with the same methodology used for plasma samples. Imatinib, imatinib-D₄ internal standard, and CGP 74588 were graciously provided by Novartis Pharmaceuticals (East Hanover, NJ). Concentrations of imatinib and CGP 74588 in plasma, tumor, and brain were determined by calculating the ratio of the area of the imatinib or CGP 74588 peak to the area under the internal standard peak in each sample and then comparing that ratio to a concomitantly performed standard curve made up in the appropriate matrix. Specifically, plasma standard curves
were prepared in plasma, tumor standard curves were prepared in a homogenate of tumor from untreated control animals, and brain standard curves were prepared in a homogenate of brain from untreated control animals.

RESULTS

Constitutive c-kit Expression and Activation in a Panel of Human SCLC Cell Lines. Constitutive expression of the receptor tyrosine kinase c-Kit has been reported in a subset of human SCLC cell lines (7–10). To determine the SCLC cell lines most appropriate for xenografts studies, protein lysates were prepared from a panel of 13 human SCLC tumor-derived cell lines and analyzed by anti-c-Kit immunoblot. Consistent with previous studies, 5 of the 13 (38%) SCLC cell lines demonstrated constitutive expression of c-Kit, with H526 and H889 exhibiting the highest levels (Fig. 1). The SCLC lines H209 and H1607 constitutively expressed c-Kit at more modest levels, and H2107 exhibited only faint c-Kit expression. Constitutive c-Kit protein expression was not detected in the SCLC lines H82, H69, H187, H378, H524, H2107, H2171, and H2227 (c-Kit was faintly detected in H69 in some experiments at longer exposure times; data not shown). Equivalent loading of SCLC protein lysates samples was confirmed by anti-actin immunoblot (Fig. 1, bottom panel).

Activation of c-Kit is accompanied by receptor tyrosine autophosphorylation (6). Thus, to assess constitutive activation of c-Kit in SCLC cell lines, protein lysates were also analyzed by anti-phosphotyrosine immunoblot. As anticipated, the SCLC cell line H526 demonstrated prominent c-Kit tyrosine phosphorylation upon stimulation (S) with its ligand SCF (Fig. 1, far right). However, the constitutive (without supplemental SCF) tyrosine phosphorylation of c-Kit in H526 and the other c-Kit-expressing SCLC cell lines, however, was quite low, detectable only at threshold levels by immunoblot (Fig. 1, middle panel). To investigate this issue further, c-Kit was immunoprecipitated from three constitutively c-Kit-expressing SCLC cell lines (H209, H526, and H1607) and analyzed by anti-phosphotyrosine and anti-c-Kit immunoblot. All three SCLC cell lines demonstrated constitutive c-Kit tyrosine phosphorylation, with H1607 demonstrating the highest relative c-Kit activation (Fig. 2A). However, as observed in the Western immunoblot analysis (Fig. 1), the constitutive tyrosine phosphorylation of c-Kit was far less than that observed when SCLC cells (H526) were stimulated with SCF (Fig. 2A, far right).

The tyrosine kinase inhibitor imatinib mesylate has been demonstrated to inhibit the SCF-induced tyrosine phosphorylation of c-Kit in SCLC cell lines in a dose-dependent manner (24, 25). However, the effect of imatinib on the constitutive tyrosine phosphorylation of c-Kit in SCLC cell lines has not been established. Therefore, H209, H526, and H1607 cells were treated overnight with various concentrations of imatinib or control, and the tyrosine phosphorylation of c-Kit was assessed by anti-phosphotyrosine immunoblot. In H526 and H1607 cells, a decrease in c-Kit tyrosine phosphorylation was detectable at imatinib concentrations as low as 0.1–0.2 μM but was more
pronounced when imatinib concentrations reached 0.3 μM (Fig. 2B). H209 cells also showed a dose-dependent decrease in constitutive c-Kit tyrosine phosphorylation with imatinib, but this was not as striking until imatinib concentrations reached 0.4–0.5 μM. At 1 μM imatinib, the constitutive tyrosine phosphorylation of c-Kit was barely detectable in all three SCLC cell lines and was completely inhibited when imatinib concentrations reached 3 μM (Fig. 2B, far left). These findings are consistent with previous studies using imatinib to inhibit SCF-induced c-Kit activation in SCLC cells in vitro (24, 25) and demonstrate that imatinib effectively inhibits the constitutive tyrosine phosphorylation of c-Kit in the human SCLC cell lines H209, H526, and H1607.

Evaluating Imatinib in Vivo Activity against Human SCLC Xenografts. To determine the activity of imatinib against SCLC xenografts in vivo, H209, H526, or H1607 cells were introduced into immunodeficient NCr nude (nu/nu) mice by s.c. injection. Besides their constitutive expression of c-Kit, these cell lines were chosen for study because of their high efficiency in establishing tumor xenografts and because they represented a spectrum of tumor growth rate from quite rapid (H526) to more modest (H1607). Once tumors reached 0.3–0.6 cm³, animals were begun on oral imatinib 100 mg/kg or control (water) twice a day by gavage. This dosing strategy was based on a regimen previously shown to inhibit Bcr/Abl-dependent signaling in vivo and produced therapeutic concentrations of imatinib in mice (Ref. 26; data not shown). Untreated H526 tumor xenografts grew rapidly, reaching the 2-cm³ cutoff tumor volume within 5 days after being established. However, imatinib treatment had no effect on H526 tumor growth (Fig. 3A). Tumor volume doubling calculations from this cohort and some additional mice revealed a H526 tumor doubling time of 3 days in imatinib-treated tumors and 3 days in controls (Table 1). H209 control-treated xenografts reached 2 cm³ after ~7 days. Imatinib produced some modest growth inhibition of H209 tumor xenografts (Fig. 3B), with final tumor volumes ~35% smaller than control-treated tumors. The tumor doubling time of imatinib-treated H209 xenografts was 4.8 days, compared with 4.0 days in control-treated tumors, although the difference was not statistically significant (P = 0.12, Table 1). H1607 tumor xenografts grew slower than H209 or H526 xenografts, reaching a tumor volume of 2 cm³ ~11 days after being established. Imatinib had no effect on H1607 tumor growth (Fig. 3C), with imatinib-treated H1607 xenografts exhibiting a tumor doubling time of 4.7 days compared with 5.0 days in controls (Table 1). These results demonstrate that imatinib had negligible activity against c-Kit-positive SCLC tumor xenografts.

Imatinib Rapidly Reaches Therapeutic Concentrations within SCLC Xenografts. On the basis of our previous study in BALB/c mice (28), it was anticipated that imatinib would reach plasma concentrations in SCLC tumor-bearing mice sufficient for inhibition of c-Kit; however, the pharmacokinetics of imatinib penetration into solid tumor xenografts was unknown. To address this question, imatinib-treated SCLC tumor-bearing mice were euthanized when tumors reached the size limit, and concomitant plasma and tumor samples were obtained for pharmacokinetic analysis. As expected, imatinib was rapidly absorbed after oral administration, reaching an average concentration of 3,841 ng/ml (6.5 μM) 2 h after a dose of imatinib of 100 mg/kg at steady state (Fig. 4; Table 2). After 8 h, plasma imatinib concentrations were still in the therapeutic range, with an average imatinib concentration of 596 ng/ml (1 μM). Interestingly, imatinib rapidly reached therapeutic concentrations in SCLC tumors (Fig. 4), with an average intratumoral imatinib concentration of 2218 ng/ml (3.8 μM) 2 h after an oral dose of
Imatinib as Targeted Therapy for Small Cell Lung Cancer

Table 1 Tumor doubling times of small cell lung cancer xenografts in imatinib- or control-treated mice

The average tumor doubling time of each cohort, plus or minus the SE, was calculated according to the formula described in “Materials and Methods.” n denotes number of tumors analyzed in each group.

<table>
<thead>
<tr>
<th>Small cell lung cancer line</th>
<th>Imatinib (days)</th>
<th>Control (days)</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H526</td>
<td>3.0 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>10</td>
<td>0.71</td>
</tr>
<tr>
<td>H209</td>
<td>4.8 ± 0.5</td>
<td>4.0 ± 0.2</td>
<td>12</td>
<td>0.12</td>
</tr>
<tr>
<td>H1607</td>
<td>4.7 ± 0.2</td>
<td>5.0 ± 0.3</td>
<td>9</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Table 2 Pharmacokinetics of imatinib in small cell lung cancer tumor-bearing mice

Average (n = 3/time point) concentrations of imatinib at the indicated times after a dose of 100 mg/kg at steady state, plus or minus the SE.

<table>
<thead>
<tr>
<th>Hours</th>
<th>Plasma ng/ml</th>
<th>Plasma µM</th>
<th>Tumor ng/ml</th>
<th>Tumor µM</th>
<th>Brain ng/ml</th>
<th>Brain µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3841 ± 537</td>
<td>6.5 ± 0.9</td>
<td>2218 ± 235</td>
<td>3.8 ± 0.4</td>
<td>109 ± 19</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>1662 ± 390</td>
<td>2.8 ± 0.7</td>
<td>1155 ± 32</td>
<td>2.0 ± 0.05</td>
<td>45 ± 4</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>8</td>
<td>596 ± 226</td>
<td>1 ± 0.4</td>
<td>372 ± 101</td>
<td>0.6 ± 0.17</td>
<td>6.5</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>51 ± 5</td>
<td>0.1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 4 Imatinib rapidly achieves therapeutic drug concentrations in small cell lung cancer (SCLC) tumor xenografts. Nude nude mice with established SCLC tumor xenografts were treated with imatinib at a dose of 100 mg/kg twice/day by oral gavage. Tumors were harvested at the indicated times after the last dose of imatinib and paired with plasma samples obtained from the same animals. Data are presented as the average imatinib concentration at steady state from three SCLC tumor-bearing mice at each time point (2, 4, 8, 15 h after imatinib). Error bars indicate the SE.

DISCUSSION

Advances in our understanding of protein-tyrosine kinases have identified an increasingly complex array of potential targets for cancer therapy (reviewed in Ref. 31). As the number of targeted cancer agents increases, one of the most important challenges in the future will be to devise strategies for identifying those signaling pathways most critical in the pathogenesis of distinct tumors. In the case of SCLC, previous studies had identified constitutive expression of c-Kit and its ligand SCF in a significant proportion of human tumor lines and primary specimens, suggesting the presence of an autocrine pathway. Although constitutive c-Kit expression was detected in some members of our SCLC tumor cell panel, the constitutive tyrosine phosphorylation of c-Kit in SCLC cell lines was generally modest, particularly when compared with SCF ligand-dependent activation (Fig. 2A). This suggested that although an SCF/c-Kit pathway might be present in selected SCLC tumor cell lines, there was relatively little constitutive c-Kit receptor activation. These results are consistent with a recent study (32) that found no evidence of activating c-Kit exon 11 mutations in a panel of human SCLC primary tumors.

Thus far, in humans, the presence of ligand-independent, constitutive tyrosine kinase activation has been a major predictor of those diseases likely to respond to imatinib. In Philadelphia chromosome-positive hematological malignancies, imatinib has shown marked activity against the constitutively activated Abl kinase (33). Chronic myeloproliferative disorders with genetic translocations, resulting in a constitutively active platelet-derived growth factor receptor have also responded well to imatinib therapy (34–36). In the vast majority of gastrointestinal stromal tumor cases, c-Kit is both overexpressed and constitutively activated (37–39), and these tumors often respond successfully to treatment with imatinib (22, 40, 41). Whether imatinib or other tyrosine kinase-targeted therapy will be successful in tumor autocrine-mediated scenarios remains to be determined.
be determined, but it is likely that the level of constitutive receptor activation will remain an important variable, as recently demonstrated in dermatofibrosarcoma protuberans tumors (42). Interestingly, in contrast to SCLC tumor cell lines in culture, the level of constitutive c-Kit tyrosine phosphorylation in control SCLC xenografts was just barely at the level of detection despite large-scale anti-c-Kit immunoprecipitation (data not shown). This limited our ability to determine whether imatinib-inhibited constitutive c-Kit tyrosine phosphorylation in vivo and suggests that the growth environment of SCLC tumor cell lines may influence the degree of constitutive c-Kit activation.

Despite the limited activity of imatinib in our SCLC xenografts, it is conceivable that imatinib therapy may still have a potential role in selected SCLC cases. It is interesting that one of our SCLC tumor cell lines (H209; Fig. 3B) showed a slight decrease in tumor growth rate with imatinib therapy, although the impact of imatinib on tumor cell doubling did not reach statistical significance (P = 0.12). In the mouse, imatinib is fairly rapidly cleared from the plasma, requiring oral administration at least twice a day, compared with once-daily imatinib dosing in humans (43). Previous studies have demonstrated that imatinib inhibits SCF-induced c-Kit autophosphorylation at an IC50 of 0.1 μM (15, 44). In SCLC cell lines, imatinib inhibited constitutive c-Kit tyrosine phosphorylation at concentrations as low as 0.1–0.2 μM in vitro, but complete inhibition of c-Kit tyrosine phosphorylation did not occur until imatinib concentrations reached 1 μM (Fig. 2B). In our mouse SCLC xenograft experiments, nadir intratumoral imatinib concentrations were 0.1 μM at the end of the longer p.m. to a.m. dose interval. Thus, it is possible that this brief period of lower imatinib concentrations could lead to an underestimation of imatinib anti-SCLC activity, particularly if higher and more constant imatinib concentrations were required to suppress SCF/c-Kit-dependent proliferation or survival.

Recently, c-Kit expression has been demonstrated to be a negative prognostic factor in SCLC patients (17), suggesting that a greater understanding of c-Kit-dependent and c-Kit-independent signaling mechanisms in SCLC will provide important insights into SCLC pathogenesis. Modulations of signal transduction pathways downstream of c-Kit and the chemokine receptor CXCR4 have been demonstrated to mediate cytoskeletal changes in human SCLC cell lines in vitro (45). Because tumor metastasis was not observed in our SCLC xenograft model, we cannot rule out that modulation of c-Kit-dependent signaling with imatinib might alter SCLC cell motility or metastasis in humans.

The rapid achievement of therapeutic imatinib concentrations in SCLC tumor xenografts was encouraging and suggests that adequate delivery of this drug to human SCLC tumors will be achievable. Our findings also demonstrate the utility of measuring intratumoral concentrations of targeted therapeutic agents to confirm that the drug reaches its intended destination and in sufficient amounts. Despite seemingly adequate delivery of imatinib to tumor tissue, the modest constitutive activation of c-Kit in SCLC suggests that imatinib will be best tested in combination with other therapeutic agents. It is conceivable that treating SCLC tumors with chemotherapy or another targeted agent might alter SCLC cell vulnerability to imatinib. Alternatively, imatinib-induced blockade of SCF/c-Kit could enhance the effects of chemotherapy. The very low imatinib concentrations in brain (Table 2) contrasted sharply with the delivery of imatinib to SCLC xenografts but are consistent with recent studies (28, 46) of poor penetration of imatinib into the cerebrospinal spinal fluid and limited distribution of imatinib to the brain (30).

In summary, imatinib treatment effectively decreased the constitutive activation of c-Kit in human SCLC cell lines in vitro. Although imatinib reached therapeutic concentrations in SCLC tumor xenografts in vivo, it had little, if any, effect on tumor growth or viability. These results suggest that the SCF/c-Kit pathway in SCLC does not mediate critical proliferative or survival signals and that therapeutically targeting this pathway alone is unlikely to be successful in treating patients with SCLC. The outcome of combination trials of imatinib with other agents in SCLC, however, will be of considerable interest.

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