In Vivo Eradication of Human BCR/ABL-Positive Leukemia Cells With an ABL Kinase Inhibitor

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Background: **The leukemia cells of approximately 95% of patients with chronic myeloid leukemia and 30%–50% of adult patients with acute lymphoblastic leukemia express the Bcr/Abl oncoprotein, which is the product of a fusion gene created by a chromosomal translocation [(9:22) (q34;q11)]. This oncoprotein expresses a constitutive tyrosine kinase activity that is crucial for its cellular transforming activity. In this study, we evaluated the antineoplastic activity of CGP57148B, which is a competitive inhibitor of the Bcr/Abl tyrosine kinase.** ***Methods:*** **Nude mice were given an injection of the Bcr/Abl-positive human leukemia cell lines KU812 or MC3. Tumor-bearing mice were treated intraperitoneally or orally with CGP57148B according to three different schedules.** ***In vitro*** **drug wash-out experiments and** ***in vivo*** **molecular pharmacokinetic experiments were performed to optimize the** ***in vivo*** **treatment schedule.** ***Results:*** **Treatment schedules administering CGP57148B once or twice per day produced some inhibition of tumor growth, but no tumor-bearing mouse was cured. A single administration of CGP57148B caused substantial (>50%) but short-lived (2–5 hours) inhibition of Bcr/Abl kinase activity. On the basis of the results from** ***in vitro*** **wash-out experiments, 20–21 hours was defined as the duration of continuous exposure needed to block cell proliferation and to induce apoptosis in these two leukemia cell lines. A treatment regimen assuring the continuous block of the Bcr/Abl phosphorylating activity that was administered over an 11-day period cured 87%–100% of treated mice.** ***Conclusion:*** **These data indicate that the continuous block of the oncogenic tyrosine kinase of Bcr/Abl protein is needed to produce important biologic effects** ***in vivo*****. [J Natl Cancer Inst 1999;91: 163–8]**

Chronic myeloid leukemia (CML) is caused by the acquisition of the reciprocal (9:22) (q34;q11) chromosomal translocation (Philadelphia chromosome) in hematopoietic stem cells. In the resulting fusion protein, part of the ABL gene product (Abl), including the tyrosine kinase (TK) domain, is fused to the amino-terminal end of the BCR gene product (Bcr) *(1)*. The Bcr domain probably interferes with an intramolecular Abl inhibitory loop and unveils a constitutive kinase activity that is absent in the normal Abl protein *(2)*. This chromosomal defect is seen in about 95% of all patients with CML as well as in 30%–50% of adult patients with acute lymphoblastic leukemia (ALL). The only curative treatment available at present is allogeneic bone marrow transplantation, a toxic and costly procedure, available for only 30% of patients. While the precise mechanism employed by the Bcr/Abl fusion protein to transform cells is still unknown, it is well accepted that the enhanced TK activity is crucial to its transforming ability *(3)*. Thus, the inhibition of the TK activity of this protein represents a specific therapeutic strategy for Bcr/Abl-expressing leukemias. A 2-phenylaminopyrimidine derivative named CGP57148B *(4)* has been reported to selectively inhibit the kinase activity of both Abl and Bcr/Abl *(5)*. CGP57148B is a competitive inhibitor of the adenosine triphosphate (ATP)-binding cleft of Abl. We and others *(6,7)* have previously reported the selective block of proliferation in BCR/ABL-positive cells (either cell lines or fresh tumor samples) and showed that this compound can commit these cells to apoptosis without inducing differentiation. So far, no data are available on the *in vivo* activity of CGP57148B on human Bcr/Abl-positive leukemia cells; results obtained in murine cells transfected with v-abl or bcr/abl indicate the presence of a limited activity, with retardation of tumor growth but no cure of treated animals *(4,5)*.

We describe here the *in vivo* effects of this compound in nude mice given an injection of human leukemia cell lines. The nude mouse model was selected because the tumor grows as a solid mass; this fact permits us to obtain purified tumor cells from animals and to assess the *in vivo* kinetics of Bcr/Abl inhibition following the treatment of tumor-bearing mice. Other models, like the severe combined immunodeficient (SCID) and SCID/non-obese diabetic (NOD) mice, permit transplantation of fresh, chronic-phase CML cells *(8)*. In this case, however, no solid tumor growth or massive outgrowth of leukemia cells in the bone marrow usually occurs, thus preventing the possibility of performing the above-mentioned experiments.

Materials and Methods

CGP57148B

A 2-phenylaminopyrimidine derivative with a molecular weight of 590, CGP57148B was developed and provided by Novartis Inc. (Basel, Switzerland). The stock solutions of this compound were prepared at 1 and at 10 m*M* with distilled water, filtered, and stored at −20 °C. For *in vitro* experiments, CGP57148B was thawed before the experiment was started and used at a concentration of 0.1–10 μ*M*. Preparations used for animal experiments were made in concentrations as indicated below and were kept at 4 °C for a maximum of 4 days.

Cell Lines

Three human leukemia lines were used: KU812, MC3, and U937. KU812 and MC3 expressed ^{p210}Bcr/Abl, whereas U937, a BCR/ABL-negative human leukemia cell line, served as a negative control *(9,10)*. Both Bcr/Abl-positive cell lines were derived from CML patients in blast crisis, who previously had been treated with busulfan. All cells were cultured in RPMI-1640 medium (BioWhittaker,

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See ''Notes'' following ''References.''

Inc., Walkersville, MD; Boerhinger Ingelheim Pharmaceuticals, Inc., Ridgefield, CT) containing 10% fetal calf serum (FCS) (HyClone Laboratories, Inc., Logan, UT) under standard cell culture conditions. Four additional Bcr/Abl-positive cell lines [KCL22, BV173, K562, and LAMA84 *(6)*] were tested in preliminary experiments, but they failed to produce reproducible growth in nude mice and were not studied further.

Determination of *In Vitro* Proliferative Activity (Tritiated Thymidine Uptake Assay)

Two hundred microliters of each cell line (KU812, MC3, and U937), containing 4×10^4 cells, was seeded at various concentrations of CGP57148B, ranging from 0.1 to 10 μ*M* in 96-well microtiter plates (Corning Costar Corp., Cambridge, MA) in six replicates. After 54 hours at 37 °C, 20 μL of RPMI-1640 medium/10% FCS containing tritiated thymidine (1 μCi/well) was added to each well. After an additional 18 hours, cells were harvested and transferred to a filter (Spot-on filtermat; Pharmacia Biotech Europe, Brussels, Belgium). Tritiated thymidine uptake was determined by a 1205 betaplate liquid scintillation counter (Wallac Inc., Turku, Finland). IC_{50} was defined as the concentration of compound producing a 50% decrease in proliferation in comparison with untreated controls.

In Vitro Wash-Out Experiments

To determine the minimal duration of drug exposure, 0.5×10^6 cells were incubated in medium containing 1 μ*M* of CGP57148B. Cells were first seeded in 24-well plates (Corning Costar Corp.). At various time points, the wells were washed twice in RPMI-1640/10% FCS and reseeded in a new 24-well plate. Each sample was seeded in six replicates. After a total culture time of 54 hours, the samples were washed again, 200 μL containing 4×10^4 cells was transferred to a 96-microtiter plate (Corning Costar Corp.), and proliferative activity was determined over a period of 18 hours (*see above*).

Western Blot Analysis

Tumor-bearing mice were treated either orally (160 mg/kg) or intraperitoneally (50 mg/kg) with CGP57148B as described below. At various time points, ranging between 2 hours and 24 hours after treatment, mice were killed and non-necrotic tumor tissue was extracted and homogenized in a fivefold volume of sodium dodecyl sulfate (SDS)-loading buffer (50 m*M* Tris–HCl [pH 6.8], 2% SDS, and 5% β-mercaptoethanol). After sonication for 2 minutes, samples were centrifuged at 15 000*g* for 15 minutes, heated at 95 °C for 10 minutes, and stored at −80 °C. Polyacrylamide gel electrophoresis was carried out on a 7.5% SDS gel (Mini-Protean II electrophoresis cell; Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were loaded as determined by BCA Protein Assay (Pierce Chemical Co., Rockford, IL). Before loading, 0.05% of bromophenol blue was added to each sample. Samples were subsequently transferred to a nitrocellulose membrane (Hybond Super-C; Amersham Life Science Inc., Arlington Heights, IL). Transfer was carried out overnight at 4 °C in a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad Laboratories) in 25 m*M* Tris, 192 m*M* glycine, and 20% methanol. After protein transfer, the membrane was blocked with TBST (0.01 *M* Tris, 0.15 *M* NaCl, and 0.05% Tween 20 [pH 7.4]) containing 5% milk, and a monoclonal anti-phosphotyrosine antibody (05–321; Upstate Biotechnology, Lake Placid, NY) was used in a 1 : 500 dilution (TBST/5% milk) to detect phosphorylated proteins. After stripping (2% SDS, 62.5 m*M* Tris–HCl [pH 6.8], and 0.7% β-mercaptoethanol for 1 hour), the filter was blotted with an anti-Abl antibody (clone Ab-3; Calbiochem Corp., La Jolla, CA; 1 : 100 in TBST/5% milk) that also recognized Bcr/Abl. An anti-mouse horseradish peroxidase-conjugated antibody (Bio-Rad Laboratories) was used as a secondary antibody and identified both primary antibodies after addition of a supersignal chemiluminescent substrate (Pierce Chemical Co.). Densitometric analysis of films was carried out on an Eagle Eye II Photodensitometer (Stratagene, La Jolla, CA). Band intensities were calculated as areas; nonspecific changes caused by differences in Bcr/Abl content (as evidenced by the anti-Abl antibody) were subtracted.

In Vivo Administration of CGP57148B

Seven to 9-week-old female CD-1 nu/nu mice purchased at Charles River Breeding Laboratories (Calco, Italy) were kept under standard laboratory conditions according to the guidelines of the National Cancer Institute, Milan, Italy. This study was approved by the institutional ethics committee for laboratory animals used in experimental research. Both Bcr/Abl-positive and -negative cell lines were injected (50×10^6 cells per animal) subcutaneously in the left flank. Treatment was started 1–8 days after leukemia cell injection. Oral treatment was administered through a syringe connected to a soft plastic tube introduced into the mouse esophagus (gavage). CGP57148B was prepared at concentrations of 16 mg/mL (oral) and 5 mg/mL (intraperitoneal) in distilled water. Control animals were given equal volumes of water. Tumor weight (TW) and total weight were monitored every 3–4 days. TW was calculated by the formula TW (mg) = $(d^2 \times D)/2$, where d and D are the shortest and longest diameters of the tumor, respectively, measured in millimeters.

When mice were treated twice per day, six mice per treatment or control group were used (total of 12 mice). For treatment of mice 24 hours after KU812 leukemia cell injection, eight mice per treatment or control group (total of 24 mice) were used, whereas the U937 group consisted of 10 mice per treatment or control group (total of 30 mice). In experiments with measurable tumor-bearing mice, 12 mice per treatment or control group (total of 24 mice) were used.

Statistical Analysis

Statistical analysis of tumor weights was performed with one-way variance analysis using the Statpac analysis program (version 3.1; Walonick Assoc., Minneapolis, MN). For survival analysis, data were compared by the logrank test *(11)*. P values <.05 were considered statistically significant and were derived from two-sided statistical tests.

Results

In Vitro Sensitivity to CGP57148B

The *in vitro* IC_{50} for CGP57148B was determined by a proliferation assay. It was found to be between 0.1 and 0.3 μ*M* in the two BCR/ABL-positive cell lines KU812 and MC3, whereas the BCR/ABL-negative cell line U937 was unaffected by CGP57148B concentrations 30 times higher (10 μ*M*) (not shown). These data are in accordance with previously published results *(6)*.

In Vitro Wash-Out Experiments

CGP57148B is a competitive (reversible) inhibitor of Abl kinase activity. To determine the minimum time of exposure to this compound necessary to inhibit proliferation, experiments were performed *in vitro* on KU812 cells. Cells were cultured in the presence of 1 μ*M* CGP57148B for the various times indicated in Fig. 1. The results clearly indicate that 20–21 hours were sufficient to block cell proliferation, whereas 6–7 hours produced little change. These data are in agreement with the kinetics of early apoptosis induction in these cell lines, which occurred between 16 and 24 hours *(6)*. Therefore, the duration of Bcr/Abl inhibition following treatment with CGP57148B could be critical in determining biologic results *in vivo*.

In Vivo Molecular Pharmacokinetic Experiments

In our previous *in vitro* experiments *(6)*, inhibition of proliferation and apoptosis induction was observed in leukemia lines when the Bcr/Abl TK activity was inhibited by 50% or more. To investigate the degree and duration of Bcr/Abl inhibition *in vivo*, we acutely treated tumor-bearing mice and killed them at various time points after treatment. The levels of Bcr/Abl kinase activity (measured as autophosphorylation) obtained at different times in a representative experiment are presented in Fig. 2. CGP57148B was administered both intraperitoneally (50 mg/kg) and orally (160 mg/kg). At 2 hours after injection, Bcr/Abl inhibition of 64.7% (intraperitoneal) and 66.4% (oral) was present in both groups. At 5 hours, the level of inhibition was still 46.7% in orally treated mice and 53.4% in intraperitoneally treated animals; by 8 hours, more than 70% of the initial kinase activity was restored in both groups. Therefore, Bcr/Abl was blocked *in vivo*, but this

inhibition was short-lived, and a single CGP57148B dose was clearly insufficient to maintain a substantial Bcr/Abl inactivation for the time necessary to block proliferation and to commit cells to apoptosis.

In Vivo Activity of CGP57148B

Since a dose of 50 mg/kg (one intraperitoneal injection/day) was used *in vivo* with murine cells *(4,5)*, this schedule was selected initially for our study and was administered for 25 days. This dosage achieves maximum concentrations (Cmax) greater than 3 μM (data not shown). No statistically significant retardation in tumor growth was observed in animals treated once per day. For this reason, the treatment schedule was modified, and the same dose was administered two times daily, at 8 AM and 4 PM. The results obtained in mice given an injection of KU812 or MC3 cells and treated twice per day are reported in Fig. 3. Tumor growth inhibition was present and reached statistical significance on days 17 ($P<.01$ for both cell lines), 21 ($P<.01$ for KU812 and $P<.05$ for MC3), and 25 ($P<.05$ for both cell lines); growth inhibition was present during the treatment period (days 1–25), while growth curves tended to run in a parallel way when the treatment was discontinued. No growth inhibition was noted in mice given an injection of the myeloid Bcr/Abl-negative cell line U937 (data not shown). The twice-per-day treatment schedule appeared necessary to inhibit tumor growth, since the administration of the same total dose (100 mg/kg intraperitoneal) in a single daily injection did not produce a statistically significant delay in tumor growth (data not shown).

These data indicate that the treatment administered at 50 mg/kg by intraperitoneal injection twice per day caused a specific and statistically significant inhibition in the growth of Bcr/Abl-positive human leukemia cell lines. However, no differences in the number of animals developing tumors or in the leukemia-free survival of treated mice were observed. Therefore, the treatment schedule was modified further, on the basis of the results from the *in vitro* wash-out and *in vivo* kinetics experiments, with the aim of producing a continuous inhibition of the Bcr/Abl kinase activity. Experiments were focused on KU812, which produced a higher *in vivo* growth rate (80%–90% of

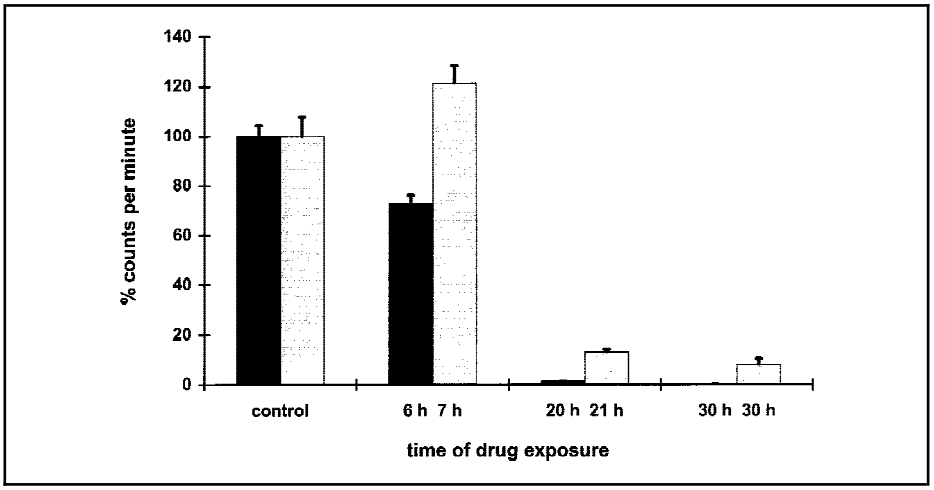

Fig. 1. Effect of different exposure times to 1 μM CGP57148B on the *in vitro* proliferation of KU812 leukemia cells. Two representative experiments (black and gray columns) are shown. Cells (5×10^5) were first seeded in 24-well plates (Corning Costar Corp.) and incubated with the drug. At the indicated time points, the cells were washed twice with RPMI-1640 medium/10% fetal calf serum and reseeded in a new 24-well plate. After a total culture time of 54 hours, the cells were washed again, and 200-μL aliquots containing 4×10^4 cells were transferred to a 96-well microtiter plate (Corning Costar Corp.) and used for an 18-hour tritiated thymidine uptake assay. Proliferative activity is expressed as the percent of control counts per minute and represents the mean of six replicates seeded for each sample. Error bars represent 95% confidence intervals (CIs). Average counts per minute for the control samples were 102 056 (95% CI = 97 546–106 566) and 39 687 (95% CI = 36 642–42 732), respectively. h = hours.

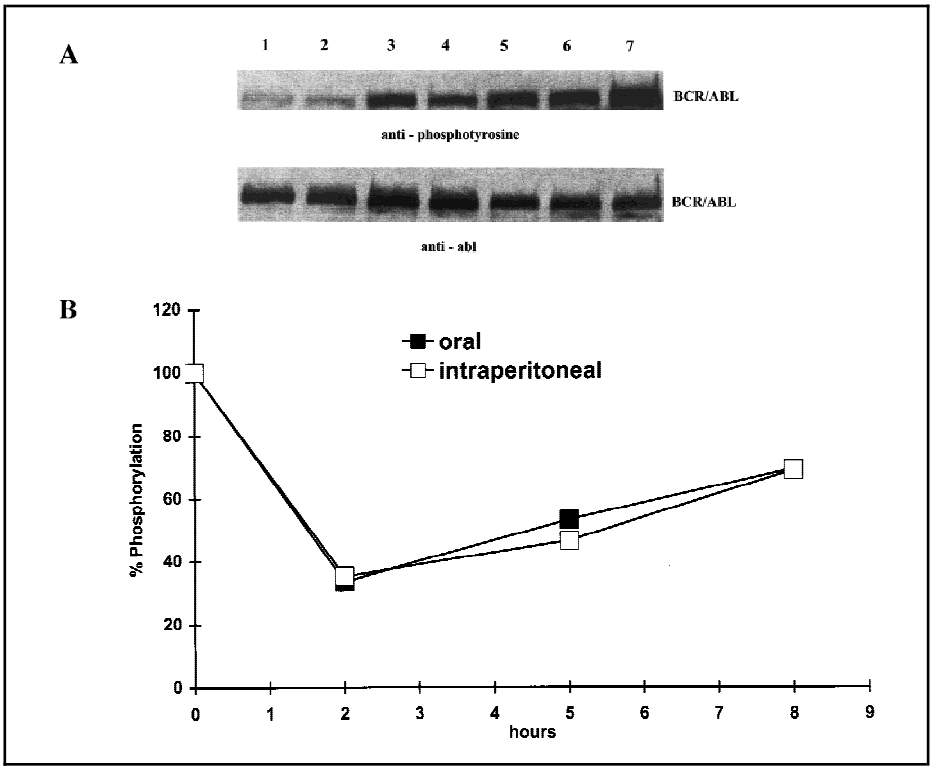

Fig. 2. *In vivo* inhibition of Bcr/Abl kinase activity by CGP57148B. Tumor-bearing mice were acutely treated with CGP57148B intraperitoneally (50 mg/kg) or orally (160 mg/kg) and killed at various time points. Tumor samples were extracted and used for western blot analysis with anti-phosphotyrosine or anti-Abelson (abl) antibodies. **A) Lane 7** represents results from an untreated mouse. **Lanes 2, 4, and 6** represent results from intraperitoneally treated animals at 2, 5, and 8 hours after treatment, respectively. **Lanes 1, 3, and 5** represent results from orally treated mice at 2, 5, and 8 hours after treatment, respectively. Western blots were first exposed to an anti-phosphotyrosine antibody and then stripped and probed with an anti-abl antibody. **B)** Photodensitometric analysis of bands labeled with the anti-phosphotyrosine antibody in **panel A.** Band intensities were calculated as areas and presented as the percentage of the time 0 value (i.e., **lane 7** in **panel A**); changes caused by differences in Bcr/Abl content (as evidenced by the control anti-abl antibody) were subtracted from the total values.

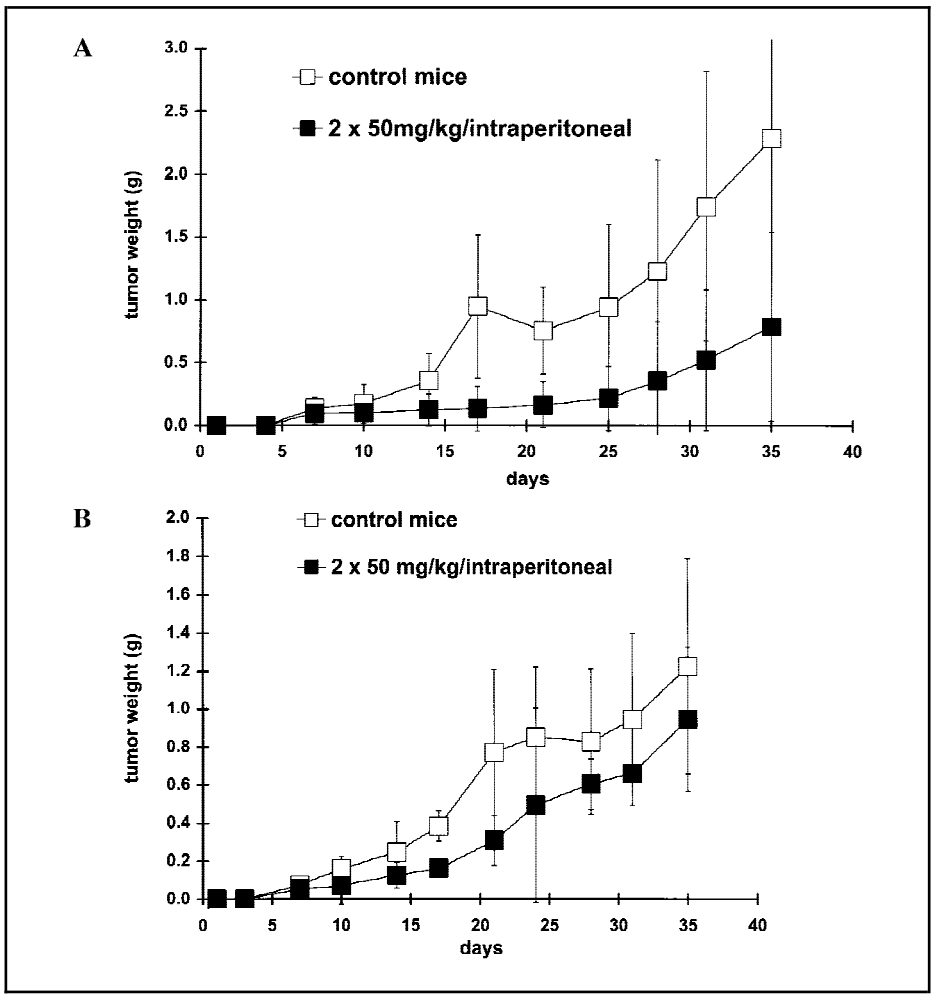

Fig. 3. Effect of CGP57148B on the *in vivo* growth of KU812 **(A)** and MC3 **(B)** Bcr/Abl-positive leukemia cell lines. CGP57148B was administered at 50 mg/kg body weight per day intraperitoneally in two daily injections (8 AM and 4 PM), 24 hours after the subcutaneous injection of 50×10^6 leukemia cells. Control mice were treated with equivalent volumes of water instead of the drug. Each experimental group contained six mice. Results are presented as average tumor weight (g); error bars represent 95% confidence intervals and are displayed only when they exceed 5% of the respective mean.

inoculated animals) than MC3 (60%–70%). Tumor-bearing mice received CGP57148B at a dose of 50 mg/kg intraperitoneally or 160 mg/kg orally every 8 hours for 11 consecutive days. Fig. 4, A, shows the tumor-free survival in animals given an injection of KU812 cells; two independent experiments were performed with similar results. Each group contained eight animals. Seven mice (87.5%) in the control group developed tumors within 9 days, while one (12.5%) of eight mice in the intraperitoneally treated group developed a tumor (87.5% were cured). All mice in the group treated orally remained tumor free. Differences in tumor-free survival between control and treated groups were highly significant ($P<.0001$), whereas the comparison between the two treated groups did not reveal statistical significance. No evidence of tumor growth was noted during the follow-up of tumor-free animals (up to 240 days). In contrast, no differences were noted between control mice and treated mice receiving an injection of U937 cells, confirming the specificity of the results obtained (not shown).

Treatment of Mice With Measurable Tumors

To study the effect of this treatment schedule on animals with more advanced disease, already bearing measurable tumors, we administered CGP57148B (oral schedule) to a group of 12 animals, 8 days after leukemia cell injection, with tumors already weighing 286 mg (95% confidence interval [CI] = 201–371 mg) in the controls and 289 mg (95% CI = 209–369 mg) in the treatment group. Fig. 4, B, presents the results of this experiment. Nodules started to regress 48 hours after treatment; the reduction, compared with the control group, averaged 68% at day 3 and 98% by day 7 after the beginning of CGP57148B treatment. No treated animal had a measurable tumor by day 8, while 12 of 12 control mice had growing nodules ($P<.0001$). The same schedule was also tested in U937-bearing mice; the treatment did not cause any statistically significant change in tumor growth or leukemia-free survival in animals given an injection of this Bcr/Abl-negative leukemia cell line (data not shown). Tumor-free animals were also followed up in this group: Four (33%) of 12 animals developed a relapse between day 48 and day 60, while the remaining eight animals (67%) have remained tumor free up to the present day (+210 days). A longer treatment schedule (18 days) apparently failed to reduce the risk of relapse (data not shown).

These data indicate that the continuous inhibition of Bcr/Abl was needed to produce major biologic effects and converted an apparently ineffective molecule (when administered once per day) to a highly active compound. However, the tumor load present at the time of treatment represented an important variable, and the treatment of mice with a measurable tumor mass, although highly effective, did not eradicate the disease in 100% of cases, as it consistently did in the group of animals treated 24 hours after the injection of leukemia cells.

Toxicity of Treatment

The weight of the animals in the treated groups increased at a reduced pace during treatment compared with the weight of the controls. This difference in weight never exceeded 8% and disappeared after treatment. No statistically significant differences in white blood cell and platelet counts or in hemoglobin levels were noted during treatment. Treated animals were also subjected to histopathologic analysis. No major finding was noted at autopsy; in particular, no changes or altered myeloid/erythroid ratios were observed in bone marrow (skull + femurs) specimens. The only abnormality observed in some, but not all, treated animals was represented by a modest periportal lymphocyte infiltrate, with no sign of hepatocellular necrosis (data not shown).

DISCUSSION

CGP57148B is a potent inhibitor of the Bcr/Abl kinase and acts by blocking the

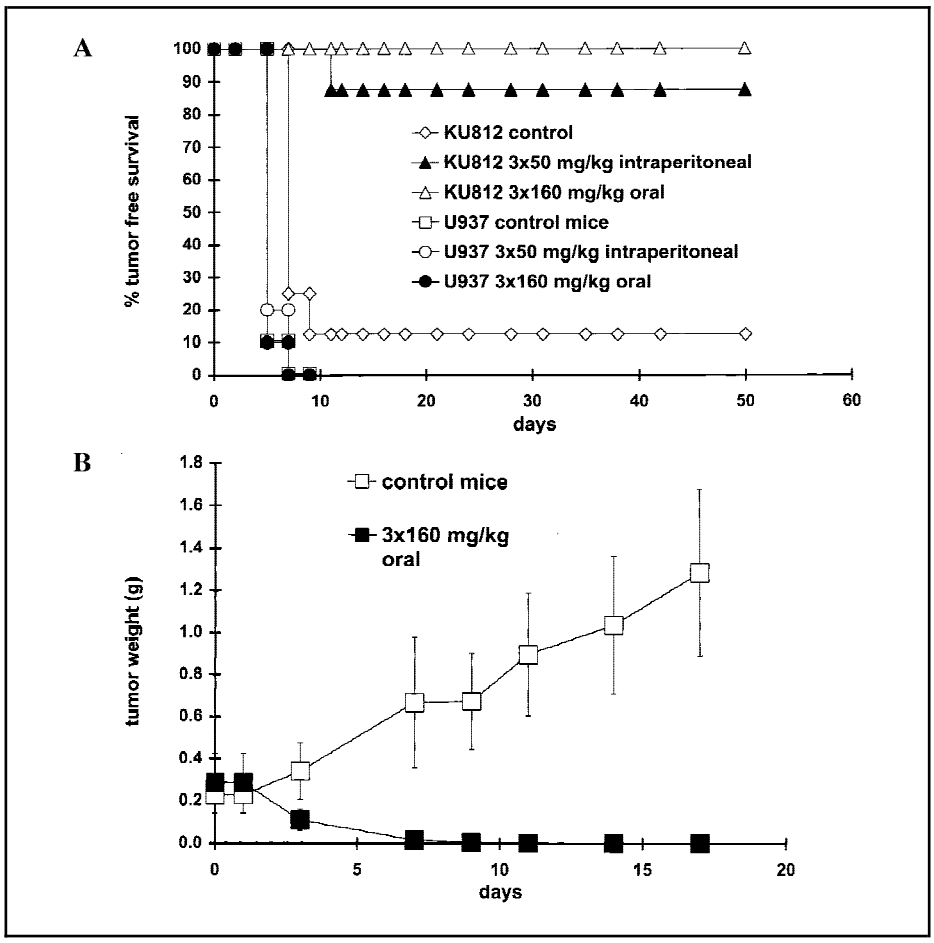

Fig. 4. Eradication of leukemia growth in mice treated with CGP57148B. **A)** Nude mice were given an injection of 50×10^6 KU812 cells on day 0. After 24 hours, treatment with CGP57148B (50 mg/kg body weight intraperitoneally or 160 mg/kg body weight orally, every 8 hours) was started and continued for 11 days. The tumor status was checked every 3–4 days. Survival data in the six groups were compared by the logrank test. KU812 treatment or control groups had eight mice per group (total of 24 mice), whereas U937 treatment or control groups had 10 mice per group (total of 30 mice). **B)** Effect of CGP57148B on leukemia growth in the presence of more advanced, measurable disease. Nude mice received an injection of human leukemia KU812 cells as described in **panel A.** Eight days later (day 0 on the *x* axis), treatment with CGP57148B (160 mg/kg body weight orally every 8 hours, for 11 days) was started. Pretreatment mean tumor weights were 286 mg (95% confidence interval [CI] = 201–371 mg) in the control group and 289 mg (95% CI = 209–369 mg) in the treatment group, respectively. Each treatment or control group had 12 mice per group.

ATP-binding site of this protein. In this report, we investigated the *in vivo* effects of CGP57148B in a nude mouse model. This model was chosen since the availability of solid nodules permits us to study the Bcr/Abl activity in tumor tissues and to assess the kinetics of Bcr/Abl inhibition following CGP57148B administration. This need would render some important experiments described here impossible to perform in the SCID/NOD model.

Our results suggest that a too short *in vivo* inhibition of Bcr/Abl is responsible for the initial negative data. The development of a treatment schedule designed to maintain a prolonged *in vivo* Bcr/Abl inactivation produced a statistically significant increase in the activity of this compound, and long-term remissions in tumor-bearing animals were achieved. The number of neoplastic cells present at the beginning of treatment seems to play an important role; in fact, when treatment was started at day 8 (in the presence of approximately 300 millions cells), one third of the animals had a relapse, while the same regimen eradicated the disease in 100% of treated animals when the treatment was started 24 hours after the injection of 50 million cells. It will be interesting to study the possible emergence of resistance to this new class of specific antineoplastic compounds. Progressive resistance to CGP57148B treatment can theoretically develop over time as a result of different mechanisms, such as cellular modifications inside leukemic cells and *in vivo* induction of drug metabolism or of proteins able to bind CGP57148B and to decrease its bioavailability. The investigation of this phenomenon bears clinical relevance, since chronic treatment with CGP57148B will probably be needed over extended periods of time.

The toxicity of the treatment was rather limited in our experience, even if the normal Abl protein was certainly inhibited [in addition to the known cross-activity with kit and pdgfrβ (i.e., platelet-derived growth factor receptor-β) *(4)*]. These results indicate that, while Bcr/Abl clearly identifies a critical ''bottleneck'' for Bcr/Abl leukemia cells, the normal Abl protein appears to be less critical for the survival of normal cells. Longer and more exhaustive toxicologic studies are, however, needed to better assess the possible long-term side effects of this molecule.

An additional point that deserves discussion is the apparent activity of the ''once-per-day'' schedule when cytokine-dependent murine cell lines transfected with v-ABL or BCR/ABL were used, as previously described *(4,5)*. In these reports, the growth of transfectants was inhibited (although no animal was cured) by a schedule that was completely ineffective in our model. However, we observed that, in these transfectants, the *in vitro* IC_{50} for CGP57148B was lower than that in the human leukemia cell lines used in our study (0.001 μM versus 0.1–0.3 μM [data not shown]), suggesting that the murine transfectants could be inhibited at CGP57148B concentrations lower than those active on KU812 or MC3. Therefore, in the experiments performed by Buchdunger et al. *(4)* and Druker et al. *(5)*, a single administration of the compound could result in longer lasting inhibitory concentrations than in our model.

These data indicate that CGP57148B exerts a specific activity against human Bcr/Abl-positive leukemia cells *in vivo;* given the competitive (reversible) nature of this compound, continuous exposure is necessary to produce major antileukemia effects.

The information contained in this report might help to establish therapeutic regimens in patients with Bcr/Abl-positive leukemias. Our results suggest, for example, that a single daily administration of CGP57148B (or of other molecules with a similar mechanism of action and *in vivo* half-life) could result in lim-

ited biologic and therapeutic effects. Although we do not yet know the *in vivo* kinetics of CGP57148B in humans, the data presented here indicate the importance of developing treatment regimens able to achieve continuous inhibition of the targeted kinase *in vivo.*

It has to be remembered, however, that nude mice given an injection of Bcr/Abl-positive cells present a hematopoietic system that is not affected by the leukemia, in contrast to CML patients, in whom most of the mature granulocytes derive from the leukemic clone; it is possible that CGP57148B might cause cytopenia in treated patients because of the low frequency of BCR/ABL-negative precursors in untreated CML patients. In addition, leukemia cells grow in nude mice as solid tumors, and even if they can spread and form distant metastases, they do not grow diffusely as in patients; attempts at injecting KU812 or MC3 intravenously were unsuccessful. Therefore, although this model was needed to carry out the *in vivo* kinetics experiments, it is less clinically relevant than other systems, like SCID/NOD mice given an injection of uncultured chronic phase CML cells *(8).*

Finally, these data could also become relevant for other neoplasias in which competitive TK inhibitors are presently being investigated *in vivo.*

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NOTES

Supported in part by the Italian Association for Cancer Research (AIRC, 420.198.662), the Italian Research Council (95.00842,9600225.CT04), Istituto Superiore di Sanità (881A/10), BIOMED-2 grant BMH4-CT96–0848, and EU-TMR grant BMH4-CT96–5006.

E. Buchdunger is an employee and stockholder of Novartis AG, Inc., manufacturer of compound CGP57148B investigated in this study.

We thank Dr. Giorgio Parmiani, Gian Marco Corneo, Pietro Pioltelli, and Enrico Pogliani for their helpful discussion.

Manuscript received June 16, 1998; revised November 4, 1998; accepted November 18, 1998.