α1 Acid Glycoprotein Binds to Imatinib (STI571) and Substantially Alters Its Pharmacokinetics in Chronic Myeloid Leukemia Patients[1]

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ABSTRACT

Purpose: **Imatinib (Glivec) is a potent inhibitor of bcr/abl, an oncogenic fusion protein that causes chronic myelogenous leukemia (CML). α1 acid glycoprotein (AGP) binds to imatinib with high affinity and inhibits imatinib activity *in vitro* and *in vivo* in an animal model. A pharmacokinetics analysis of imatinib was undertaken in CML patients.**

Experimental Design: **Imatinib plasma concentrations were measured in 19 CML patients treated with imatinib (400 or 600 mg/day). Five patients received a concomitant short-term course of clindamycin (CLI).**

Results: **A positive correlation between AGP and imatinib plasma levels was observed. CLI administration decreased imatinib plasma concentrations, evaluated as area under the curve (*AUC*) and peak concentrations (C_{max}). The effects of a bolus of CLI was studied in three patients on imatinib 23 h after the last imatinib dose. Within 5–10 min in three of three cases, CLI caused a decrease in imatinib plasma concentrations of 2.6-, 2.7-, and 4.7-fold, respectively. *In vitro* experiments using fresh blasts from CML patients showed that AGP, at concentrations observed in the patients, decreased imatinib intracellular concentrations up to 10 times and blocked imatinib activity. The incubation with CLI restored imatinib intracellular concentrations and biological activity.**

Conclusion: **AGP exerts significant effects of the pharmacokinetics, plasma concentrations, and intracellular distribution of imatinib in CML patients; these data indicate that plasma imatinib levels represent unreliable indicators of the cellular concentrations of this molecule.**

INTRODUCTION

Imatinib represents a specific inhibitor of the oncogenic tyrosine kinase Bcr/Abl. Preclinical (1–3) and early clinical results (4–8), show an impressive antileukemic activity of imatinib, usually in the absence of serious toxicity. Despite its excellent clinical activity, most patients affected by Ph+ acute leukemias treated with imatinib achieve only transient responses. These responses are soon followed by the development of resistance despite high imatinib plasma concentrations.

Two molecular mechanisms causing cellular resistance to imatinib have been identified: *BCR/ABL* gene amplification (9–13), and mutations in the catalytic domain of Bcr/Abl (12–16). Gene amplification and mutations have been mostly observed in patients with acute leukemia.

A third mechanism of resistance to imatinib was identified only in an animal model (17) and consists in the leukemia-related induction of an acute phase protein called AGP.[3] AGP binds imatinib with high affinity and blocks its biological activity (proliferation and kinase activity). Drugs known to compete with imatinib for binding to AGP (18), such as erythromycin or CLI, could displace imatinib from AGP in this model, restoring its biological and therapeutic activity.

Important differences exist between this model and the clinical situation. Basal human AGPs levels are 4–5 times higher than murine ones; therefore, AGP levels can rise, after inflammatory stimuli, up to 20–30-fold over basal values in mice, and only 2–4-fold in humans. In addition, given the higher basal values in humans, "normal" levels of AGP are theoretically sufficient to bind most of the imatinib that is present in patients' plasma (17).

Independently from any direct contribution of AGP to resistance, the presence of this protein in the plasma of patients could have important effects on imatinib PK.

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[3] The abbreviations used are: AGP, α1 acid glycoprotein; CML, chronic myelogenous leukemia; PK, pharmacokinetics; BC, blast crisis; AP, accelerated phase; CP, chronic phase; TW, tumor weight; HPLC, high-performance liquid chromatography; SS, steady state.

In the present report, standard PK analysis was performed in 19 patients affected by CML and treated with imatinib. The relationship between AGP levels and PK parameters was studied, as well as the alteration in PK caused by the concomitant administration of drugs known to bind AGP.

PATIENTS AND METHODS

Patients. Nineteen patients affected by CML in BC (n = 5), AP (n = 7), or CP (n = 7) were studied after written informed consent. The patients were entered in five different registrative or non-registrative trials. Imatinib was administered p.o. in a single daily administration 2 h after a meal (usually breakfast), together with 250 ml of water. The total daily dosage of imatinib ranged between 400 and 600 mg. Five patients concomitantly received CLI for the treatment of established infections or for prophylaxis. CLI was infused as a continuous i.v. infusion of 2.7 g/day, preceded by a bolus injection of 900 mg over 20 min, on the first day of treatment. This schedule is recommended by the manufacturer to produce plasma CLI concentrations of >15 μM.

Blood samples for PK analysis were obtained on day 1, and/or after SS was reached (day 4–29). Citrated samples were collected at time 0, and at 30 min and 1, 2, 3, 4, 8, and 24 h after dosing. Blood samples were centrifuged at 800 × *g* at 4°C for 5 min; plasma samples were stored at 4°C and analyzed within 3 days.

Additional samples were collected as heparinized (50 units/ml) samples; blood was centrifuged (400 × *g* for 30 min at 15°C) on a ficoll gradient, and mononuclear cells recovered between the ficoll and the plasma layers. Cells were washed twice in cold PBS (BioWhittaker Europe, Verviers, Belgium) and resuspended in RPMI + 10% FCS or frozen.

Mice. Seven-to-9-week-old female CD1 nu/nu mice purchased from 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. KU812 BCR/ABL-positive leukemic cells were injected (50×10^6 cells/animal) s.c. into the left flank of the animals. Imatinib was administered p.o. at 160 mg/kg every 8 h through a syringe connected to a soft plastic tube introduced into the esophagus (gavage). 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 the longest diameters of the tumor, respectively, measured in millimeters. Treatment was started when tumors reached or exceeded 1 g in weight.

HPLC Determination of Plasma and Tissue Imatinib Concentrations. Imatinib was determined in plasma after deproteinization of 0.5 ml of the samples with an equal volume of acetonitrile. The mixture was maintained at room temperature for 20–30 min and the protein precipitate removed by centrifugation at 13,000 rpm × 5 min. One hundred μl of the supernatant were injected into the separation module Alliance 2690 (Waters, Milford, MA) for the HPLC analyses that were carried out using an EC 125/4 Nucleosil 100-5 C18 column in line with a CC 8/4 Nucleosil 100-5 C18 precolumn (Macherey-Nagel, Düren, Germany).

The column was equilibrated at the flow rate of 1 ml/min with 10% v/v acetonitrile in water containing 0.05% trifluoroacetic acid (solution A) and then the sample was eluted using a gradient to the final condition of 90% v/v acetonitrile in water containing 0.05% v/v trifluoroacetic acid (solution B) over a period of 20 min. The column was prepared for the next sample by holding this condition for 5 min and then returning to the initial condition for 5 min. After chromatographic separation, the peak was detected at 270 nm by a Waters 2487 absorbance detector. The acquisition system was a Millennium32 software for chromatography (Waters).

To prepare the calibration curve, imatinib was added to human plasma or to tissue extract, yielding a final concentration of 2000 ng/ml. This solution was further diluted in human plasma or tissue extract to achieve analyte concentrations of 1000, 500, 250, and 100 ng/ml. Standard samples were processed as described above.

To measure the free fraction of the drug, 2 ml of the plasma samples (3-h time point) were collected into Centrifugal Filter Device Centriplus, with a cutoff of M_r 30,000 (Amicon, Millipore Corporation, Bedford, MA) and centrifuged at 3000 rpm for 50 min. A volume of 200 μl of the obtained ultrafiltrate was injected into the HPLC instrumentation under the same conditions reported above.

The limits of detection in plasma and ultrafiltrate were 100 and 50 ng/ml, respectively.

To determine imatinib tissue concentrations, organs from nude mice were extracted and homogenized in a 4-fold volume of PBS at pH 7.4 using an Ultra Turrax homogenizer (Janke and Kunkel, Ika-Werk, Germany). After homogenization for 2 min, samples were extracted with 1:2 volumes of Acetonitrile and incubated for 30 min at room temperature. Subsequently, samples were centrifuged at 13,000 rpm for 5 min, and the acetonitrile phase was collected and injected (100 μl) into the HPLC apparatus. Blanks and standard samples were prepared using tissue extract from control animals.

To obtain μM concentrations, μg/ml values must be multiplied by a factor of 1.69.

Pharmacokinetic Parameters. The experimental area under the curve of the concentration *versus* time points (*AUC*) was calculated by the linear trapezoidal rule. C_{max} values were obtained from experimental data. Clearance (*Clss*) and Volume of distribution (*Vss*) at the SS were defined according to the following equations that can be used during repeated oral administration regimen: $Clss = \text{Dose} * F/(Css * \tau)$ and $Vss = Clss/K_e$ (19), where F is the bioavailability of the drug, τ is the dosing interval, K_e is the elimination constant, and *Css* is the average plasma concentration at SS. *Css* was calculated as: $Css = AUCss/\tau$, where *AUCss* is the area under the curve within a dosing interval at SS (19).

Because we do not know the bioavailability of imatinib, but we need only an intrapatient comparison of the parameters, the clearance and the volume of distribution were calculated as: $Clss/F = \text{Dose}/(Css * \tau)$ and $Vss/F = Clss/K_e$ (19).

The percentage of free imatinib and free imatinib concentrations were experimentally measured by ultrafiltration, as described above.

AGP Determination. AGP levels were detected by immunodiffusion, using an antibody specific for human AGP

(Cardiotech Services Inc., Louisville, KY), as described previously (17).

Chemicals. Imatinib was provided by Novartis Pharma AG, Basel, Switzerland. It is a derivative of a 2-phenylaminopyrimidine, with a M_r of 590. For *in vitro* experiments, stock solutions of imatinib were prepared at 1 and 10 mM in distilled water, filtered, and stored at −20°C.

CLI was used as CLI base (Sigma Chemical Co., St. Louis, MO). CLI was dissolved in ethanol and then diluted 1:1000 in distilled water and used. For clinical use, CLI was used as a 20% solution (Clindamicina Ibi) and diluted in saline immediately before use.

Determination of the *in Vitro* Cell Proliferation Activity ([^{3}H]Thymidine Uptake Assay). Six to eight replicate cultures (200 μl), each containing 10^4 cells, were incubated with 0–10 μM imatinib in 96-well microtiter plates (Corning Costar Corp., Cambridge, MA) for 54 h at 37°C. After this period, 20 μCi of RPMI-1640 (BioWhittaker Europe) and 10% FCS containing [^{3}H]thymidine at a dose of 1 pCi/well (DuPont NEN, Boston, MA) were added to each well. After an additional 18 h, cells were harvested and transferred to a filter (Printed Filtermat; Wallac Oy, Turku, Finland. [^{3}H] Thymidine uptake was measured in a 1205 betaPlate liquid scintillation counter (Wallac Inc., Turku, Finland). The IC_{50} inhibitory concentration (defined as the concentration of a compound producing a 50% decrease in proliferation compared with untreated controls) was calculated.

Statistical Analysis. Statistical analysis was performed with Student's *t* test by use of the GraphPad software analysis program (Prism, San Diego, CA). *P*s of less than 0.05 were considered to be statistically significant and were derived from two-sided statistical tests. All of the data are presented as the mean (±95% confidence interval). Confidence intervals are displayed when they exceed 10% of the respective mean. Correlation Coefficient (*CC*) was calculated using the Pearson method.

RESULTS

Imatinib PK. The initial PK of imatinib was investigated in 13 subjects from whom day 1 plasma samples were available. Eight patients received a dose of 400 mg and five a dose of 600 mg.

Fig. 1 shows the plasma concentration-time curves determined in the eight patients who received 400 mg of the drug. After administration, imatinib C_{max} was achieved between 1 and 3 h; then in all of the patients, the drug was slowly cleared from plasma, being still detectable at 24 h. Table 1 reports the main pharmacokinetic parameters of imatinib determined in all of the 13 patients studied at day 1. In the patients treated at 400 mg, mean C_{max}, experimental 24-h *AUC*, and half-life were 2.35 ± 1.0 μg/ml, 24.66 ± 8.5 μg/ml h, and 12.5 ± 2.4 h, respectively. The five patients treated at 600 mg showed mean C_{max}, *AUC*, and half-life of 7.83 ± 3.8 μg/ml, 99.74 ± 54.1 μg/ml h and 12.5 ± 1.4 h, respectively. Differences in *AUC* and C_{max} between patients treated with 400 and 600 mg were statistically significant. For this reason, a correlation between imatinib dose (expressed as mg/m^2) and plasma levels was performed. The interval of mg/m^2 ranged from 200 to 471 mg/m^2. A positive correlation existed with both C_{max} ($r = 0.70$, $P = 0.01$) and *AUC* ($r = 0.76$, $P = 0.01$).

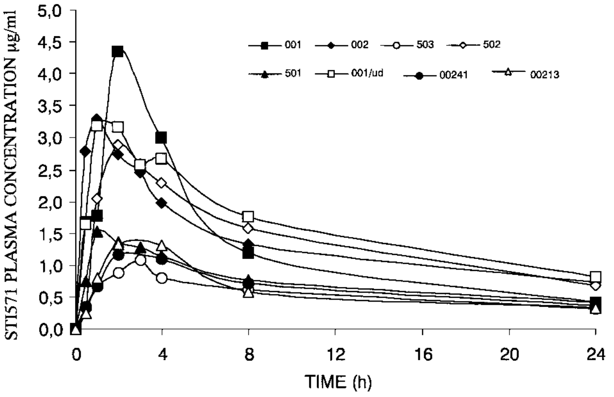

Fig. 1 Imatinib plasma concentrations determined in eight patients treated at 400 mg on day 1. Symbols refer to individual patients.

At SS (day 4 to 29), drug accumulation was noted, with C_{max}, *AUC*, and half-life (400-mg dose level) of 4.45 ± 2.0, 57.0 ± 18.9, and 16.6 ± 5.9, respectively; a positive correlation between dose and *AUC* ($r = 0.57$, $P = 0.001$) or C_{max} ($r = 0.54$, $P = 0.005$) was also observed.

Because AGP is known to bind imatinib with high affinity (17), we evaluated the relationship between AGP levels and imatinib plasma concentrations. AGP values in the patients studied ranged between 0.36 and 1.80 mg/ml. A significant linear correlation was found ($r = 0.686$, $P < 0.01$, $n = 19$) between C_{max} at SS (normalized at the dose of 300 mg/m^2) and AGP plasma levels (Fig. 2). A similar trend was also noted between AGP and *AUC*, although it did not reach statistical significance.

To evaluate the displacing effect of CLI on the AGP/imatinib complex, five patients who received a concomitant infusion of CLI while on imatinib (400 mg/day) were studied. The duration of CLI administration varied between 24 h and 5 days, depending on the medical indication (*i.e.*, treatment *versus* prophylaxis of infection). CLI was administered as an initial 900 mg i.v. bolus (over 20 min.) followed by 2.7 g/day continuous infusion. Patients had PK performed at SS (day 4), 24 h before the start of CLI administration. The initial bolus administration of CLI coincided with a new PK study (day 5), which was then compared with the previous one. As can be seen in Fig. 3, the administration of CLI dramatically reduced imatinib plasma concentrations. It is important to note that imatinib concentrations obtained at 30 min decreased, compared with time 0, although patients received a new dose of imatinib at time 0. Table 2 reports the PK data and the binding of imatinib to plasma proteins obtained before and after CLI administration. On day 4, mean C_{max} and AUC were of 4.72 ± 3.3 μg/ml and 57.49 ± 23.1 μg/ml h, respectively. After the coadministration of the antibiotic, the means of C_{max} and *AUC* were 1.43 ± 1.0 μg/ml and 19.50 ± 5.5 μg/ml h, respectively, which was approximately three times lower than those obtained on day 4. All of these comparisons between data obtained at day 4 and during CLI administration were statistically significant. In addition, a significant increase of *Clss* and *Vss* were observed, suggesting a higher tissue distribution of imatinib, after CLI administration.

CLI was able to reduce the mean protein bound drug from

Table 1 Main PK parameters of imatinib in CML patients on day 1 of treatment

Patient	Disease phase	Dose (mg)	$T_{max}{}^{a}$ (h)	C_{max} (μg/ml)^{b}	*AUC* (μg/ml h)^{c}	$t_{1/2}$ (h)
001	BC	400	2	4.35	32.99	7.3
002	BC	400	1	3.29	27.80	15.2
0503	AP	400	3	1.08	13.20	14.2
0502	AP	400	2	2.89	34.00	11.4
0501	AP	400	1	1.53	18.00	12.8
001/udine	AP	400	1.5	3.18	40.40	12.7
00213	CP	400	2	1.33	15.10	13.1
00241	CP	400	2	1.17	15.80	13.5
		Mean		2.352	24.66	12.5
		95% CI		1.05	8.50	2.4
0504	AP	600	3	6.21	82.60	13.7
002/udine	AP	600	4	6.19	87.70	13.5
003	BC	600	1	4.80	44.90	10.6
004	BC	600	4	12.15	158.90	12.8
003/udine	AP	600	3	9.82	124.58	11.4
		Mean		7.83	99.74	12.5
		95% CI		3.80	54.15	1.4

[a] T_{max} indicates the time (h) to peak concentrations.
[b] C_{max} values were obtained from experimental data.
[c] *AUC*s were calculated by trapezoidal rule.

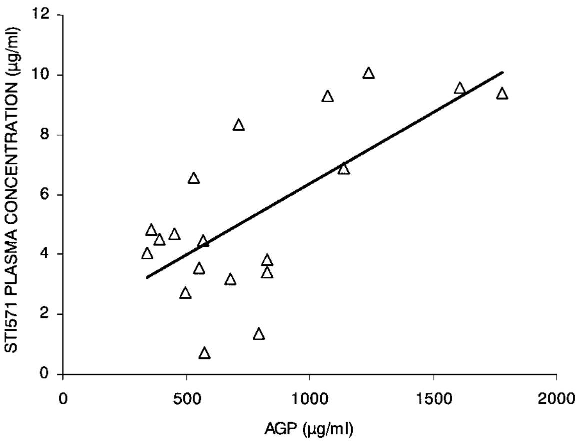

Fig. 2 Correlation between AGP levels and plasma imatinib concentrations (C_{max}, SS). AGP values were determined on the same day of pharmacokinetic evaluation.

99.0 ± 1.1% to 96.0 ± 2.4%. The amount of free imatinib (expressed as μg/ml) increased from 0.035 ± 0.02 to 0.051 ± 0.02 ($P = 0.06$), suggesting an increase in free drug concentration. This increase was of course much lower than the observed decrease in total plasma imatinib, because of the rapid distribution of the free drug into the extravascular volume.

To further study this phenomenon and to exclude effects mediated by imatinib metabolism, three patients were studied after the infusion of the initial bolus of 900 mg CLI. In these patients the administration of CLI started 23 h after the last dosing of imatinib. The results are presented in Table 3 and show a substantial decrease in imatinib plasma concentrations immediately after the infusion of CLI in all three patients.

Therefore, the infusion of CLI caused a rapid and significant decrease in imatinib plasma concentrations with increased distribution of the drug and a tendency to increased free-drug concentrations.

Effect of CLI Administration on Tissue Imatinib Concentrations in Mice. The above reported results indicate that a substantial portion of imatinib is bound to AGP in the plasma of patients. The administration of another molecule that competes with imatinib for AGP binding was soon followed by a decrease in plasma levels. It can be expected that the release of imatinib from AGP increases drug tissue distribution. This assumption cannot be experimentally verified in patients for obvious ethical reasons. Therefore, the experiment was performed on tumor-bearing nude mice; animals were treated with imatinib at 160 mg/kg three times a day as described previously (17). The fourth dose was coadministered with 350 mg/kg CLI, as described previously (17). Eleven animals were used in each group. Mice were killed 2 h after the last dose and were quickly subjected to pathological analysis. Organs (liver, spleen, intestine, kidney, tumor) were extracted and analyzed for imatinib content. The results are presented in Table 4. Increases in imatinib levels were observed in all of the organs examined, including the tumor. The extent of increase ranged from 49% (intestine) to 106% (liver), and was statistically significant in all of the organs examined with the exception of the intestine. Similar results were obtained when erythromycin was used in place of CLI, at the same dose (data not shown).

Biological Effects of AGP on Leukemic Cells Isolated from Patients. To document the biological effects of AGP on responsiveness to imatinib, blasts were tested *ex vivo*. Cells

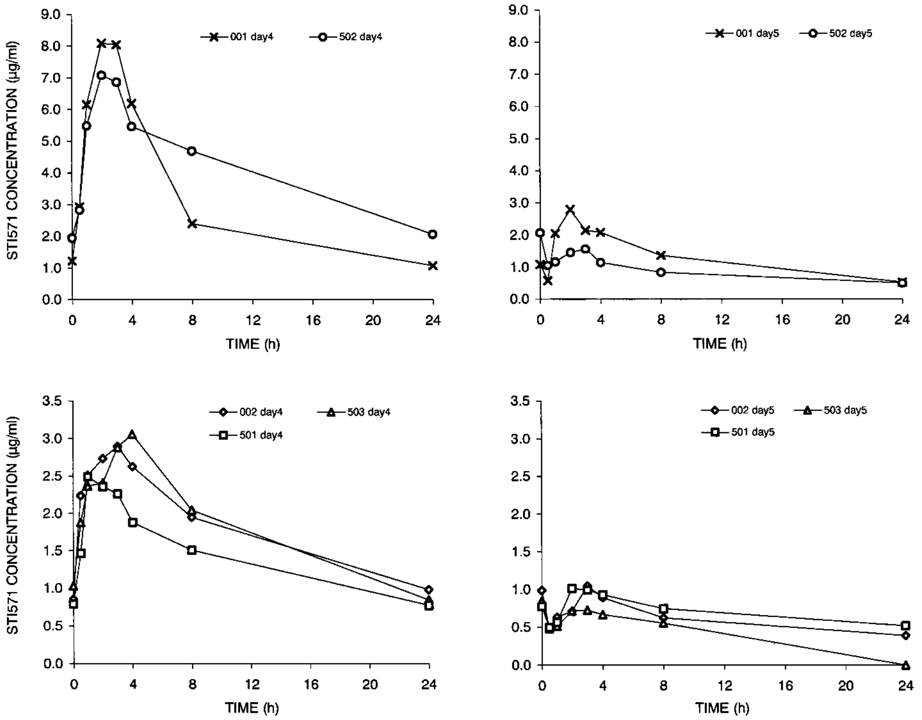

Fig. 3 Effect of CLI administration on SS imatinib plasma levels in five patients. Time refers to hours after the start of CLI infusion (day 5) and after the administration of the daily dose of imatinib (day 4 and day 5). Symbols refer to individual patients.

Table 2 Effect of CLI on the PK parameters of imatinib and on its binding to plasma proteins

	Day 4[a]						Day 5[f]					
Patient	C_{max}[b] (µg/ml)	*AUC*[c] (µg/ml h)	*Clss*[d] (liter/h)	*Vss*[d] (liter)	% bound	Free imatinib[e] (µg/ml)	C_{max} (µg/ml)	*AUC* (µg/ml h)	*Clss* (liter/h)	*Vss* (liter)	% bound	Free imatinib (µg/ml)
001	8.09	70.47	5.68	84.7	99.5	0.04	1.79	30.20	13.25	172.6	98.5	0.052
002	2.90	42.70	9.37	185.2	97.4	0.0654	1.04	14.30	27.97	585.3	93.8	0.0645
0503	3.06	43.20	9.26	152.3	99.2	0.0245	0.73	16.59	24.11	434.9	94.6	0.0394
0502	7.08	97.70	4.09	86.3	99.5	0.0354	1.57	20.00	20.00	519.5	95.5	0.0707
0501	2.49	33.38	11.98	266.8	99.5	0.0125	1.02	16.40	24.39	876.4	97.6	0.0245
Mean	4.72	57.49	8.08	155.1	99.02	0.035	1.43	19.50	21.94	517.7	96.0	0.051
95% CI	3.30	23.15	2.77	66.6	1.07	0.02	1.02	5.54	4.93	223.1	2.41	0.019

[a] Day 4 refers to PK performed at SS.
[b] C_{max} values were obtained from experimental data.
[c] *AUC*s were calculated by trapezoidal rule.
[d] Clearance (*Clss*) and the volume of distribution (*Vss*) were calculated as described in "Patients and Methods."
[e] The percentage of free imatinib and free imatinib concentrations were experimentally measured by ultrafiltration and HPLC determination.
[f] Day 5 refers to PK performed during CLI infusion, 24 h after the day 4 PK.

were incubated for 1 h with imatinib, imatinib + AGP, or imatinib + AGP + CLI. Subsequently, cells were either pelleted and assessed for intracellular concentrations of imatinib or seeded in 96-well plates and assessed for sensitivity to imatinib (4). Table *5* presents the results obtained from patient 001 (in whom blasts made up 95% of the cells used in this experiment), representative of three patients tested. It is evident that imatinib at 3 µM blocked, almost completely, the proliferation of blasts. AGP at 1.5 mg/ml (the levels experimentally determined in this patient) decreased the intracellular concentration of imatinib to less than 10% of control and almost completely abrogated the biological activity of imatinib (evidenced here as inhibition of proliferation). The addition of CLI partly restored both the intracellular concentrations of imatinib and its biological activity (inhibition of proliferation).

DISCUSSION

Imatinib induces remissions in Ph+ leukemias with high frequency and minimal toxicity. However, most patients with acute leukemias experience only transient responses, and the majority of patient in CP remain PCR-positive[4] (20) despite

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[4] C. Gambacorti, unpublished observations.

Table 3 Effect of the administration of CLI (900 mg i.v. over 20 min) on imatinib plasma concentrations (μg/ml)

Three patients at SS received an i.v. infusion of CLI (900 mg over 20 minutes), 23 h after the last dose of imatinib. Five and 10 min after the end of CLI infusion, blood samples were obtained and total imatinib concentrations assessed.

Patient no.	Imatinib dosage	Disease phase	Pre-CLI	5 min post-CLI	10 min post-CLI
008	600	BC	7.85	1.66	1.68
00213	400	CP	0.32	0.12	0.12
00241	400	CP	0.35	0.14	0.13

Table 4 Imatinib distribution in murine organs

Tumor-bearing nude mice were treated with imatinib (160 mg/kg) with or without CLI (350 mg/kg) p.o. Two h after dosing, animals were killed and organs extracted.

	Imatinib (160 mg/kg)		Imatinib (160 mg/kg) + CLI (350 mg/kg)		
Organ	Mean (μg/g)	95% CI	Mean (μg/g)	95% CI	P
Liver	49.6	±18.7	101.3	±35.6	<0.01
Spleen	74.5	±24.3	132.3	±44.1	<0.01
Kidney	49.3	±17.6	100.8	±41.5	0.01
Intestine	49.1	±24.1	69.7	±26.5	0.06
Tumor	30.3	±10.8	57.8	±13.7	0.03

Table 5 Effects of AGP and CLI on intracellular concentrations and proliferative activity of leukemic blasts from patient 001

	Imatinib intracellular concentrations (ng/ml)[a]	Proliferation ([^{3}H]thymidine uptake)[b]
Control	0	45,926 ± 2,622
Imatinib	3,492 ± 417	1,581 ± 281
Imatinib + AGP	162 ± 49	43,879 ± 4,159
Imatinib + AGP + CLI	843 ± 151	3,160 ± 831
AGP	0	41,518 ± 4,740
CLI	0	48,861 ± 3,157

[a] Blasts (1.5 × 10^8) were incubated with imatinib (3 μM) and/or AGP (1.5 mg/ml) and CLI (20 μM) for 1 h at 37°C. Subsequently cells were centrifuged at 400 × *g* for 10 min at 4°C; the supernatant was accurately discarded, and the pellet was frozen immediately. Imatinib concentrations were determined on the pellet by HPLC.

[b] An aliquot of cells was seeded, before centrifugation, in 96-well plates and the proliferative activity assessed after 54 h of culture by [^{3}H]thymidine uptake assay.

complete cytogenetic responses. The reasons for the lack of eradication of the neoplastic clone are probably multiple (9, 12, 17, 21) and incompletely understood. An apparent paradox is constituted by the fact that resistant patients show plasma concentrations that are apparently active *in vitro* on their own leukemic cells (22). A hypothesis to explain this finding could reside in the inability of the drug present in plasma to distribute to tissues and to penetrate the target CML cells. Consistent with this hypothesis are the findings shown in the present study; in fact a relationship between AGP and plasma concentrations of imatinib was established.

The experiments conducted with CLI, an antibiotic that binds AGP *in vitro* and can displace imatinib bound to AGP, further corroborated this view. In fact, it was found that the simultaneous administration of CLI induced substantial alterations in imatinib PK, including a rapid and dramatic decrease in total plasma levels, decreased $C_{\max}$ and *AUC* values. This rapid decrease in imatinib plasma levels just after CLI administration indicates that the interaction is not related to the induction of imatinib metabolism but it is related to an increase in the protein-free fraction of imatinib and increased tissue distribution. This hypothesis, suggested by the finding that *Vss* is increased after CLI administration (Table 2), was verified in mice, in which it was shown that CLI caused an increase in imatinib levels in neoplastic and normal tissues.

These data also indicate that plasma concentrations of imatinib that are measured in patients are not a reliable indicator of the concentrations reached inside the leukemic cells, which represent the ultimate target for imatinib activity.

A recent report questions the ability of AGP to bind imatinib (23). However, a number of technical and methodological differences (24) render it difficult to compare the results obtained in that study with the ones of the present and other reports on this issue (17, 22, 25, 26). Briefly, Jörgensen *et al.* (23) never used our preparation of AGP as control, thus rendering a formal comparison not possible. In addition, the authors state in their paper that our AGP preparation, supplied by Sigma, "risks desialylation of the protein." However, the authors fail to acknowledge that such phenomenon has been associated with a decrease (or to no change at all) in drug binding and not in an increased binding, as their data apparently suggest.

For most drugs, the displacement of protein binding does not significantly affect drug exposure and pharmacological effect because the transient changes in free-drug concentration are rapidly equilibrated (27). However, in the case of imatinib, we have previously shown that, at least in an animal model, the presence of high levels of AGP in plasma strongly reduces the antileukemic activity of the drug and that the displacement by erythromycin (17) restored the therapeutic activity. In theory, the displacement of imatinib from AGP by erythromycin might make imatinib more available for metabolism or elimination mechanisms. If this were the case, a decrease in the antileukemic activity of imatinib by concomitant treatment with erythromycin would be expected. Instead, we observed, at least in CML-bearing mice, an increase of antitumor activity, thus suggesting that erythromycin-induced displacement of imatinib from AGP causes an increase of the drug distribution in leukemic cells. Alternatively, one could hypothesize that the increase in imatinib free fraction results in an increased formation of metabolites that are more potent than the parent compound. This hypothesis needs to be further verified, although, thus far, there is no evidence that metabolites of imatinib that are more potent than the unchanged drug exist.

Although we showed that AGP influences imatinib PK and tissue distribution, we do not propose that AGP directly causes resistance to imatinib in patients; rather we suggest that the main effect of AGP is to "artificially" increase the plasma level of the drug and to render it a poor marker for intracellular concentrations. Our results show that the free imatinib levels marginally rose after AGP displacement (0.035 to 0.051; see "Results").

Such a marginal increase probably had a therapeutic activity in the mouse model (17), in which no major cellular resistance to imatinib was present, but will probably have a less evident activity in the human situation, in which much higher cellular heterogeneity and longer selection times are present.

These data can also provide an explanation, in resistant patients, for the presence of plasma imatinib concentrations that are active *in vitro* against the leukemic cells derived from the same patients. Plasma concentrations evidently contain two different forms of imatinib: the one bound to AGP that is not distributed in tissues, and the one not bound to AGP that is potentially active. The net effect of such a phenomenon is that the majority of imatinib present in plasma is bound to AGP and not biologically active. In fact, the mere incubation of blood samples, derived from patients on imatinib treatment but showing resistance to it, with erythromycin [another drug known to displace imatinib from AGP (17)] *in vitro* was able to restore the biological activity of imatinib on Bcr/Abl autophosphorylation (22).

Approximately 99% of imatinib was found to be protein bound. After CLI administration, the fraction of protein-bound imatinib decreased in a statistically significant way, increasing the free fraction by 4-fold (from 1% to 4%). However, >90% of plasma imatinib remained protein bound, even during CLI infusion. The decrease in total concentrations was much more evident than the changes in free-drug concentrations. The explanation of this result is not certain; the most likely interpretation is that free imatinib rapidly leaves the intravascular compartment, whereas the remaining imatinib re-equilibrates with other proteins, like albumin. In fact albumin can bind imatinib, although with an affinity of 2.3×10^5 liters/mol, compared with 4.9×10^6 for AGP, and without affecting its ability to enter cells (17). It is important to note that the experimental conditions in which the free fraction of imatinib was measured (ultrafiltration) cannot differentiate between a strong (and biological relevant) type of binding and a weaker one.

What strategies could be adopted to overcome resistance to imatinib?

The fact that imatinib concentrations in leukemic cells are probably lower than plasma levels because of the presence of AGP, as well as previous data on detection of *BCR/ABL* gene amplification in resistant lines and patients (9–13), indicate that, in these cases, an increase in dosing could overcome drug resistance. There are, however, instances in which resistance is caused by the mutation of the catalytic domain of BCR/ABL; in such cases, the increase of dosage could theoretically be insufficient to counteract the resistance, because the mutated enzyme is no longer sensitive to the inhibitory activity of the drug. Although it is clear that mutations will require the development of imatinib analogues (if feasible), it is also true that the experimental conditions in which a selective pressure is applied can either favor or disfavor the likelihood of resistant cells to be selected. An example is represented by the LAMA84-R cell line, which harbors a clearly identifiable alteration (a marker chromosome with >14 copies of *BCR/ABL* on it) and can grow in 1 μM imatinib (9). This line could be selected only by exposing cells to "marginally active concentrations" of imatinib (slightly less than its IC_{50}) and then gradually increasing them; when active concentrations (1 μM) were used from the beginning, all cells were killed and no selection was possible. It is evident, therefore, that the exposure of leukemic cells to marginally active imatinib concentrations, which probably happens in tissues at present dosages, will favor such a selection.

Increases in imatinib dosages are presently hampered by regulatory requirements present in some countries and by the lack of parenteral formulations of imatinib; in addition, the tolerability of high dosages of imatinib has not been extensively tested.

Additional studies will be needed to translate the rapidly accumulating information on the molecular nature of imatinib resistance (9–17, 22) into better therapeutic options for patients affected by Ph+ leukemias.

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REFERENCES

1. Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., Zimmermann, J., and Lydon. N. B. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat. Med., *2:* 561–566, 1996.

2. le Coutre, P., Mologni, L., Cleris, L., Marchesi, E., Buchdunger, E., Giardini, R., Formelli, F., and Gambacorti-Passerini. C. *In vivo* eradication of human BCR/ABL-positive leukemia cells with an ABL kinase inhibitor. J. Natl. Cancer Inst. (Bethesda), *91:* 163–168, 1999.

3. Deininger, M. W., Goldman, J. M., Lydon, N., and Melo, J. V. The tyrosine kinase inhibitor CGP57148B selectively inhibits the growth of BCR-ABL-positive cells. Blood, *90:* 3691–3698, 1997.

4. Gambacorti-Passerini, C., le Coutre, P., Mologni, L., Fanelli, M., Bertazzoli, C., Marchesi, E., Di Nicola, M., Biondi, A., Corneo, G. M., Belotti, D., Pogliani, E., and Lydon. N. B. Inhibition of the ABL kinase activity blocks the proliferation of BCR/ABL+ leukemic cells and induces apoptosis. Blood Cells Mol. Dis., *23:* 380–394, 1997.

5. Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., Lydon, N. B., Kantarjian, H., Capdeville, R., Ohno-Jones, S., and Sawyers, C. L. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N. Engl. J. Med., *344*: 1031–1037, 2001.

6. Talpaz, M., Silver, R. T., Druker, B. J., Goldman, J. M., Gambacorti-Passerini, C., Guilhot, F., Schiffer, C. A., Fischer, T., Deininger, M. W., Lennard, A. L., Hochhaus, A., Ottmann, O. G., Gratwohl, A., Baccarani, M., Stone, R., Tura, S., Mahon, F. X., Fernandes-Reese, S., Gathmann, I., Capdeville, R., Kantarjian, H. M., and Sawyers. C. L. Imatinib induces durable hematologic and cytogenetic responses in patients with accelerated phase chronic myeloid leukemia: results of a Phase 2 study. Blood, *99:* 1928–1937, 2002.

7. Kantarjian, H., Sawyers, C., Hochhaus, A., Guilhot, F., Schiffer, C., Gambacorti-Passerini, C., Niederwieser, D., Resta, D., Capdeville, R., Zoellner, U., Talpaz, M., and Druker. B. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N. Engl. J. Med., *346:* 645–652, 2002.

8. Sawyers, C. L., Hochhaus, A., Feldman, E., Goldman, J. M., Miller, C. B., Ottmann, O. G., Schiffer, C. A., Talpaz, M., Guilhot, F., Deininger, M. W., Fischer, T., O'Brien. S. G., Stone, R. M., Gambacorti-Passerini, C. B., Russell, N. H., Reiffers, J. J., Shea, T. C., Chapuis, B., Coutre, S., Tura, S., Morra, E., Larson, R. A., Saven, A., Peschel, C., Gratwohl, A., Mandelli, F., Ben-Am, M., Gathmann, I., Capdeville, R., Paquette, R. L. Druker, B. J. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a Phase II study. Blood, *99:* 3530–3539, 2002.

9. le Coutre, P., Tassi, E., Varella-Garcia, M., Barni, R., Mologni, L., Cabrita, G., Marchesi, E., Supino, R., and Gambacorti-Passerini, C. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood, *95:* 1758–1766, 2000.

10. Weisberg, E., and Griffin, J. D. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood, *95*: 3498–3505, 2000.

11. Mahon, F. X., Deininger, M. W., Schultheis, B., Chabrol, J., Reiffers, J., Goldman, J. M., and Melo, J. V. Selection and characterization of BCR-ABL positive cell lines with differential sensitivity to the tyrosine kinase inhibitor STI571: diverse mechanisms of resistance. Blood, *96*: 1070–1079, 2000.

12. Gorre, M. E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P. N., and Sawyers, C. L. Clinical resistance to STI-571 cancer therapy caused by *BCR-ABL* gene mutation or amplification. Science (Wash. DC), *293*: 876–880, 2001.

13. Kreil, S., Muller, C., Lahaye, T., La Rosee, P., Corbin, A., Schoch, C., Cross, N. C., Berger, U., Rieder, H., Druker, B., Gschaidmeier, H., Hehlmann, R., and Hochhaus, A. Molecular and chromosomal mechanisms of resistance in CML patients after STI571 (Glivec) therapy. American Society of Hematology, 43rd Annual Meeting, Orlando, Florida. Blood, *98*: 435, 2001.

14. Hofmann, W. K., Jones, L. C., Lemp, N. A., de Vos, S., Gschaidmeier, H., Hoelzer, D., Ottmann, O. G., and Koeffler, H. P. Ph(+) acute lymphoblastic leukemia resistant to the tyrosine kinase inhibitor STI571 has a unique *BCR-ABL* gene mutation. Blood, *99:* 1860–1862, 2002.

15. von Bubnoff, N., Schneller, F., Peschel, C., and Duyster. J. *BCR-ABL* gene mutations in relation to clinical resistance of Philadelphia-chromosome-positive leukaemia to STI571: a prospective study. Lancet, *359:* 487–491, 2002.

16. Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., Herrmann, R., Lynch, K. P., and Hughes. T. P. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood, *99:* 3472–3475, 2002.

17. Gambacorti-Passerini, C., Barni, R., le Coutre, P., Zucchetti, M., Cabrita, G., Cleris, L., Rossi, F., Gianazza, E., Brueggen, J., Cozens, R., Pioltelli, P., Pogliani, E., Corneo, G., Formelli, F., and D'Incalci, M. Role of α1 acid glycoprotein in the *in vivo* resistance of human BCR-ABL(+) leukemic cells to the abl inhibitor STI571. J. Natl. Cancer Inst. (Bethesda), *92:* 1641–1650, 2000.

18. Kremer, J. M., Wilting, J., and Janssen, L. H. Drug binding to human α-1-acid glycoprotein in health and disease. Pharmacol. Rev., *40:* 1–47, 1988.

19. Rowland, M., and Tozer, T. Clinical Pharmacokinetics, Ed. 2, chapter 7. Philadelphia: Lea & Febiger, 1989.

20. Stentoft, J., Pallisgaard, N., Kjeldsen, E., Holm, M. S., Nielsen, J. L., and Hokland, P. Kinetics of BCR-ABL fusion transcript levels in chronic myeloid leukemia patients treated with STI571 measured by quantitative real-time polymerase chain reaction. Eur. J. Haematol., *67:* 302–308, 2001.

21. Graham, S. M., Jörgensen, H. G., Allan, E., Pearson, C., Alcorn, M. J., Richmond, L., and Holyoake, T. L. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 *in vitro*. Blood, *99:* 319–325, 2002.

22. Gambacorti Passerini, C., Rossi, F., Verga, M., Ruchatz, H., Gunby, R., Frapolli, R., Zucchetti, M., Scapozza, L., Bungaro, S., Tornaghi, L., Rossi, F., Pioltelli, P., Pogliani, E., D'Incalci, M., and Corneo, G. Differences between *in vivo* and *in vitro* sensitivity to imatinib of Bcr/Abl+ cells obtained from leukemic patients. Blood Cells Mol. Dis., *28:* 361–372, 2002.

23. Jörgensen, H. G., Elliott, M. A., Allan, E. K., Carr, C. E., Holyoake, T. L., and Smith. K. D. α1-Acid glycoprotein expressed in the plasma of chronic myeloid leukemia patients does not mediate significant *in vitro* resistance to STI571. Blood, *99:* 713–715, 2002.

24. Gambacorti Passerini, C., le Coutre, P., Zucchetti, M., and D'Incalci, M. Binding of imatinib by α1-acid glycoprotein. Blood, *100:* 367–368, 2002.

25. Larghero, J., Mahon, F., Madalaine-Chambrin, I., Raffoux, E., Faure, P., Berthaud, P., Taksin, A., Bastie, J., Dombret, H., Degos, L., Chomienne, C., and Rousselot, P. Elevated levels of the plasma protein α1 acid glycoprotein in chronic myelogenous leukemia in blast crisis mediate pharmacological resistance to Gleevec (STI571, imatinib) *in vitro* and are associated with primary resistance *in vivo*. American Society of Hematology, 43rd Annual Meeting. Orlando, Florida. Blood, *98*: 616, 2001.

26. le Coutre, P., Kreuzer, K., Na, I., Lupberger, J., Holdhoff, M., Appelt, C., Schwarz, M., Muller, C., Gambacorti Passerini, C., Platzbecker, U., Bonnet, R., Ehninger, G., and Schmidt. C. Determination of α-1 acid glycoprotein in patients with Ph+ chronic myeloid leukemia during the first 13 weeks of therapy with STI571. Blood Cells Mol. Dis., *28:* 75–85, 2002.

27. Benet, L. Z. and Hoener, B. Changes in plasma protein binding have little clinical relevance. Clin. Pharmacol. Ther., *71:* 115–121, 2002.

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