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ORIGINAL ARTICLE

A phase I study of imatinib mesylate in combination with chlorambucil in previously treated chronic lymphocytic leukemia patients

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Abstract
Purpose The tyrosine kinase inhibitor, imatinib, has the potential to indirectly inhibit DNA repair. This mechanism of action has been shown to mediate sensitization to chlorambucil in chronic lymphocytic leukemia (CLL). To evaluate this effect in vivo, we performed a phase I study of chlorambucil combined with imatinib in relapsed CLL patients.
Methods The three dose levels studied included imatinib at 300, 400, or 600 mg/day. Imatinib was given on days 1–10, and chlorambucil (8 mg/m^2 daily) was given on days 3–7 of a 28-day cycle (up to 6 cycles).
Results Eleven patients participated in this study. Low-grade gastrointestinal toxicities were observed in a dose-dependent manner. Forty-five percent of patients responded (two unconfirmed CRs and three PRs). Two responding patients were fludarabine refractory. The in vitro IC_{50} of chlorambucil alone or in the presence of 5 μM imatinib in CLL lymphocytes correlated with the decrease in lymphocyte counts on day 15. Imatinib plasma concentrations achieved in patients were in the range of those effective in in vitro sensitization studies.
Conclusion The combination of chlorambucil and imatinib in patients with previously treated CLL was well tolerated and showed evidence of clinical efficacy. Based on our results, we recommend the 400 mg daily dose of imatinib on days 1–10 with 8 mg/m^3 chlorambucil on days 3–7 every 28 days as the phase II dose. This represents the first clinical trial examining the potential synergy between a tyrosine kinase inhibitor and a conventional alkylating agent for the treatment of CLL.

Keywords Chronic lymphocytic leukemia · Chlorambucil · Imatinib · Phase I trial · Tyrosine kinase inhibitor · Alkylating agent

This manuscript is dedicated to the memory of Dr. Merrill J. Egorin.

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Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of a clonal population of mature-appearing B-lymphocytes in the blood, bone marrow, and lymphoid tissue [1]. It is more common in the elderly, who are less likely to be candidates for aggressive treatment such as multiagent chemotherapy regimens or stem cell transplant [2]. More effective oral therapies with limited toxicities are needed for such patients.

Chlorambucil is a nitrogen mustard which for many years was the treatment of choice for CLL [3]. Although newer treatment protocols have largely replaced chlorambucil in the treatment of younger and fit patients, for those who are not candidates for aggressive treatment, chlorambucil remains a reasonable first-line option [2]. Chlorambucil is an alkylating agent that binds to DNA, RNA, and many intracellular proteins. Chlorambucil induces DNA damage through binding of up to two DNA sites per chlorambucil molecule thus creating interstrand crosslinks.

This is believed to be the major mechanism of cytotoxicity in cancer cells in general as well as in CLL (reviewed in [3, 4]). Eventually, CLL becomes resistant to chlorambucil. There are several potential mechanisms of resistance; one of these mechanisms is through upregulation of DNA repair pathways [4, 5].

Interstrand crosslinks are repaired through a combination of nucleotide excision repair and homologous recombination (HR). The Rad51 molecule is a central player in homologous recombinational repair, and higher levels have been associated with resistance of cancer cells to chemotherapy [6]. One of the key regulators of Rad51 is c-Abl tyrosine kinase. c-Abl is activated in response to DNA damage and leads to phosphorylation of Rad51. This results in upregulation of Rad51, enhanced binding to Rad52 (required for repair activity), and increased binding to DNA of the Rad51 complex [7–9].

There has been interest recently in the use of tyrosine kinase inhibitors to increase the sensitivity of cancer cells to alkylating agents [10, 11]. Imatinib mesylate is a selective tyrosine kinase inhibitor with activity primarily against c-Abl, as well as c-kit and PDGFR, and is used as first-line treatment in chronic myeloid leukemia [12, 13]. Inhibition of c-Abl impairs the activity of Rad51 and thus potentially HR, ultimately leading to increased sensitivity to DNA damaging agents such as alkylators. Aloyz et al. have shown that imatinib sensitizes CLL lymphocytes to the effects of chlorambucil in vitro, in both untreated and chlorambucil-resistant samples. This was associated with imatinib-mediated inhibition of chlorambucil-stimulated c-Abl phosphorylation and subsequent decrease in chlorambucil-stimulated Rad51 phosphorylation and Rad51 foci [14]. Another group showed that imatinib sensitized glioma cells (but not normal fibroblasts) to the cytotoxic effects of radiation, and this effect was associated with decreased expression of Rad51 [15].

The present phase I study examined the tolerability of imatinib in combination with standard dose chlorambucil in adult patients with previously treated CLL. The primary objectives of this study were to determine the recommended phase 2 dose (RP2D), as well as the toxicities of imatinib in combination with chlorambucil in previously treated CLL patients. Secondary objectives included assessment of efficacy and duration of response; pharmacokinetic studies to determine peak/steady-state plasma concentrations of imatinib at each dose level; and assessment of sensitization to chlorambucil in vitro using CLL lymphocytes isolated from patients on study. We hypothesized that imatinib would sensitize CLL lymphocytes to the cytotoxic effects of chlorambucil through inhibition of c-Abl, resultant inhibition of Rad51, and the process of HR. This is the first clinical study of a tyrosine kinase inhibitor in combination with an alkylating agent in the treatment of CLL.

Materials and methods

Patient population

Patients ≥18 years old with a confirmed diagnosis of CLL as per WHO criteria and an indication for treatment according to NCI working group criteria were eligible for the study. Patients needed to have received at least one prior chemotherapy regimen, including chlorambucil or fludarabine. Any additional prior treatments, including monoclonal antibodies, corticosteroids, immunotherapies, or radiation, were permitted. Patients with a white blood cell count at time of enrollment $>25 \times 10^9$/l were sought for this study. The eligibility criteria included: an ECOG performance status of 0–2; adequate renal and hepatic function (indicated by serum creatinine ≤150 mmol/l, ALT $<2.5 \times$ upper limit of normal, total bilirubin $\leq 2 \times$ upper limit of normal); platelet count $> 75 \times 10^9$/l and transfusion independence; and neutrophil count $>1.0 \times 10^9$/l. This study was approved by Health Canada plus by the Research Ethics Board of the Jewish General Hospital and of the Charles Lemoyne Hospital, where patients were recruited. All patients gave informed consent before any study-related procedures were performed.

Patients were excluded for the following reasons: known history of seropositivity for HIV, Hepatitis B or C; active cardiovascular disease as defined by New York Heart Association (NYHA) class III–IV categorization; concurrent use of chronic steroids, except as replacement therapy for adrenal insufficiency; serious underlying medical illnesses or active infections requiring parenteral antibiotics that would interfere with the ability of the patient to carry out the treatment program; concurrent malignancy (other than resected basal or squamous cell skin cancers or in situ cervical cancer); and pregnant or breastfeeding female patients. Patients were also excluded from the study if they had received any previous therapy for CLL within 28 days prior to the study entry or were receiving any concomitant treatment for CLL except intravenous immunoglobulins.

Treatment plan and study design

Patients were enrolled sequentially at three imatinib dose levels: 300, 400, or 600 mg daily on days 1–10, and a total fixed oral dose of chlorambucil 8 mg/m^2 daily on days 3 to day 7 per 28-day cycle. Up to 6 cycles of monthly therapy were given The imatinib doses were selected based on doses shown to be well tolerated in patients with chronic myelogenous leukemia or GIST.

The selected chlorambucil dose is equivalent to the total dose used in the CALGB intergroup trial [16] and was divided over several days to enable the sensitization by imatinib without continuous exposure to either agent. Dividing the dose of chlorambucil over several days is an established dosing regimen for the treatment of CLL. Three patients were enrolled at each dose level. Dose-limiting toxicity (DLT) was defined as any ≥grade 3 toxicity by CTCAE (Common Terminology Criteria for Adverse Events) Version 3.0 in cycle 1, any toxicity that delayed therapy beyond day 35 of the first cycle, or missing three or more doses of chlorambucil, imatinib, or both in cycle 1 due to toxicity. If no DLTs occurred, three patients were enrolled at the next dose level. If one of three patients at any dose level exhibited a DLT, that dose level was expanded to six patients. If the next three patients did not exhibit DLT, treatment would be increased to the next dose level. If two patients presented with DLT at any given dose level, then the previous dose level would be designated the maximum tolerated dose (MTD) after six patients had been evaluated at that dose level and <1 DLT was observed. There was no intrapatient dose escalation. Since no dose-limiting toxicities were seen at the first two dose levels of imatinib, we proceeded to treat patients at the 600 mg dose level.

Patients were evaluated on day 1 of each cycle before receiving treatment, and as clinically indicated. A complete blood count with differential and a serum biochemistry profile (including electrolytes, BUN, creatinine, and liver enzymes) were performed weekly for the first 2 cycles and then on day 1 of all subsequent cycles. A urinalysis was performed on day 1 of each cycle.

Dose modifications and criteria for retreatment

Therapy was delayed for ≥grade 3 neutropenia or for >grade 1 thrombocytopenia. Therapy was resumed at the same dose if neutropenia and/or thrombocytopenia resolved to grade ≤2 and grade ≤1, respectively. A complete blood count (CBC) was performed at weekly intervals until these criteria were met. Doses were not changed for delays of 1 week, but a dose reduction to the next lowest dose level was required for delays lasting >2 weeks. If treatment was delayed for >4 weeks, the patient was taken off the study.

The treatment course was planned for 6 cycles. Criteria for early withdrawal from the study were as follows: disease progression; intercurrent illness preventing further administration of treatment; unacceptable adverse event(s); withdrawal of consent; or changes in the patient's condition rendering the patient unacceptable for further treatment in the judgment of the investigator.

Response evaluation

All patients underwent pre-study evaluations that consisted of a complete blood count, a physical examination (with measurements of lymphadenopathy, hepatomegaly, and splenomegaly), and a radiological examination by CT scan. Physical examination for tumor assessment, as well as $\beta 2$ microglobulin measurement, was performed at the beginning of every odd cycle. If radiological examination performed prior to starting treatment showed evidence of disease, it was repeated at every odd cycle. Response was measured according to the criteria of the 1996 NCI Working Group [17].

Statistical analysis

Patient characteristics were summarized using descriptive statistics. For the in vitro correlative studies, statistical analysis was performed using Sigmaplot, Version 10.0, 1996 (Statsoft Inc). Groups being compared were assessed for normal distribution. If they passed, analysis was done using paired *t* test, if not the Wilcoxon signed rank test was used. Correlations were assessed using the Pearson or Spearman rank correlation coefficient with r cutoff value of 0.6. In all cases, significance was considered if values were ≤0.05.

In vitro studies and pharmacokinetics

In vitro lymphocyte cytotoxicity assays using patient lymphocytes were done to assess sensitization to chlorambucil by imatinib as previously described [14]. Peripheral blood samples were collected in heparinized tubes before imatinib treatment on day 1 of cycle 1 and day 3 of cycle 1, 2 h after imatinib but prior to chlorambucil administration. B-lymphocytes were isolated as previously described (<10% t-cells) [14] and plated and incubated in the presence of various concentrations (0–100 μM) of imatinib alone (generously provided by Novartis Pharmaceuticals, Dorval, Quebec, Canada), chlorambucil (Sigma–Aldrich Canada, Oakville, Ontario, Canada) alone or in combination. The MTT assay was performed 72 h after treatment. Synergy was determined by the formula: $a/\mathrm{A} + b/\mathrm{B} = \mathrm{I}$, where a is the chlorambucil IC_{50} (the concentration that results in 50% of control) obtained in combination with imatinib at concentration b; A is the chlorambucil IC_{50} without imatinib; and B is the imatinib IC_{50} in the absence of chlorambucil. According to the formula, when $I < 1$, the interaction is synergistic, when $I = 1$, the interaction is additive, and when $I > 1$, there is an antagonistic interaction [14].

Pharmacokinetic studies of imatinib were carried out as follows: blood samples were collected in heparinized tubes in cycle 1 on days 1, 3, and 10; pre-treatment plus 2- and 4-h

post imatinib. Plasma was prepared by centrifugation and stored frozen at −70°C until analyzed for concentrations of imatinib and its major metabolite, desmethyl imatinib, by a validated LC–MS assay [18].

Results

Patient demographics

From March 2006 to August 2007, 11 patients were enrolled in this study (Table 1). Median age of the patients was 73 (range 49–86), and 6/11 were men. Every patient had received at least one prior conventional treatment for CLL, and five (45%) had been heavily pre-treated with ≥three regimens. The median number of previous treatment courses was two (range 1–4). Nine patients had previously been treated with fludarabine (See Table 1). Five patients were fludarabine refractory, as defined by CLL relapse on therapy or within 6 months of the last dose of a fludarabine-containing regimen [16]. Two patients had received only chlorambucil as prior treatment.

Table 1 Patient characteristics

	N
Total patients	11
Age (years)	
Median	73
Range	49–86
Sex	
Male	6
Female	5
Risk Category	
Rai stage I–II	7
Rai stage III–IV	4
Number of prior treatments	
Median	2
Range	1–4
Previous fludarabine-containing regimen	
1 treatment	5
>1 treatment	4
Previous Rituximab	2
Best response to most recent pre-study treatment	
CR	3
PR	6
PD	1
Unknown	1

Toxicity

Overall, the combination of chlorambucil and imatinib was well tolerated (Table 2). Treatment-related toxicities consisted mainly of gastrointestinal toxicities, intensifying in grade and frequency at the highest dose level of imatinib. The most common toxicity was nausea, with or without vomiting, which was reported in six patients. The two patients with grade 2 nausea were treated at the highest dose level of imatinib. Anorexia was also reported in six patients. Most cases of anorexia were grade 1 and occurred only in the first cycle, with the exception of a grade 3 anorexia seen in cycle 4 for a patient on the highest dose level. Hematologic toxicity was mild and was primarily observed in patients with major cytopenias at baseline. There was one DLT, grade 3 thrombocytopenia, in a patient at dose level 3. This patient was thrombocytopenic at baseline at 77×10^9/l. There was one serious adverse event (SAE) of febrile neutropenia which occurred during cycle 5 for a patient receiving the highest dose level of imatinib.

Efficacy

Three patients completed the full 6 cycles of treatment (Table 3). The median number of cycles completed was four (range 1–6). There were two patients at dose level 3 who met criteria for CR in terms of blood counts and clinical exam; however, bone marrow examination was declined, and thus, CR could not be confirmed. There were three PRs, two at dose level 1 and one at dose level 2. Overall, the response rate was 5/11 or 45%. Of note, 6/9 or 67% of evaluable patients (as defined by those patients reaching day 1 of cycle 3) experienced >50% decrease in lymphocyte counts. Two fludarabine refractory patients had a PR, and one patient with progressive disease on chlorambucil alone experienced a PR. Two additional patients had stable disease. For responders, the median progression-free survival was 14 months (Table 3). One patient who had a PR and completed all 6 cycles of treatment experienced transformation to Hodgkin's lymphoma 4 months after completing the study. Reasons for discontinuation of treatment included: completion of all 6 cycles of therapy (three patients); progression of disease (three patients); SAE (two patients); DLT (one patient); withdrawal by treating physician due to lack of response as defined by stable disease (one patient) and patient non-compliance (one patient) (Table 3).

Interestingly, as shown in Fig. 1, lymphocyte counts increased during the first week of treatment and then declined. A similar pattern was also noted for neutrophils (data not shown). The most dramatic example was a patient in dose level 3 with a baseline lymphocyte count of

Table 2 Treatment-related toxicities by grade and imatinib dose level

Cohort (imatinib dose) (mg)	No. in cohort	Category	Toxicity	No. of pts affected	Highest grade	Cycle in which toxicity first presented
300	3	Blood/bone marrow	Thrombocytopenia	1	2	C2
		Gastrointestinal	Diarrhea	1	1	C1
			Nausea	2	1	C4
					1	C5
			Vomiting	1	1	C2
		Pulmonary/upper respiratory	Cough	1	1	C1
400	3	Gastrointestinal	Anorexia	2	1	C2
					2	C1
			Nausea	1	1	C1
			Vomiting	1	1	C1
600	5	Blood/bone marrow	Anemia	2	3	C2
					2	C1
			Neutropenia	1	3	C2
			Thrombocytopenia	1	3 (DLT)	C1
		Constitutional symptoms	Fatigue	2	1	C1
					1	C2
		Lymphatics	Edema to legs	1	1	C2
		Gastrointestinal	Anorexia	4	3	C4
					1	C1
					1	C1
					1	C1
			Diarrhea	1	1	C1
			Dry mouth	1	1	C1
			Heartburn	1	2	C1
			Nausea	3	2	C1
					2	C2
					1	C1
			Taste alteration	1	2	C1
			Vomiting	2	2	C1
					1	C1
		Infection	Febrile neutropenia	1	3 (SAE)	C4
		Musculoskeletal	Weakness	1	1	C1

60.5×10^9/l, rising to 311.6×10^9/l on day 8, then decreasing to 68.1×10^9/l on Day 15, and 29.4×10^9/l on day 22. This patient went onto have a complete response (unconfirmed by bone marrow), and on day 1 of the cycle 6 had a lymphocyte count of 4.7×10^9/l.

Pharmacokinetics

Plasma imatinib concentrations were measured pre-treatment plus at 2 and 4 h after imatinib administration in cycle 1 on days 1, 3, and 10, prior to chlorambucil administration. These time points were chosen to coincide with the t_{max} for imatinib, reported as 3.1 ± 2 h in CML patients receiving 400 mg daily imatinib [19]. Pharmacokinetic data were obtained for all patients. The results for imatinib plasma concentrations in the CLL patients on day 1 and day 10 are shown in Fig. 2. The steady-state (pre-treatment) values of imatinib on day 3 (data not shown) and day 10 were ≥ 3 μM at all dose levels. Furthermore, 2–4 h after imatinib administration on day 3 and day 10, the imatinib concentration was ≥ 6 μM (Fig. 2).

Correlative studies

Patients had blood collected for in vitro lymphocyte cytotoxicity assays on day 1 prior to initiation of treatment and day 3, 2 h after imatinib and prior to chlorambucil. Samples were available for nine patients. At day one, prior to

Table 3 Efficacy analysis

Patient no.	Cohort (imatinib dose) (mg)	No. of cycles completed	Reason for withdrawal	Best response[a]	Progression-free survival (months)[b]
1	300	6	Completed all cycles	PR	9
2		4	Lack of response	SD	
3		6	Completed all cycles	PR[d]	15
4	400	3	PD	SD	
5		1	SAE (pneumonia, unrelated)	N/A	
6		5	Non-compliance	PR[d]	10
7	600	3	SAE (febrile neutropenia)	CR[c]	17
8		6	Completed all cycles	CR[c]	13
9		2	PD	PD	
10		1	DLT	N/A	
11		2	PD	PD	

[a] Response was assessed at start of cycle 3 (week 8) and start of cycle 5 (week 16)

[b] Progression-free survival (PFS) is indicated only for patients exhibiting a response (CR or PR). For patient 6, PFS was not counted in median PFS calculations since this patient refused regular follow-up visits, but did exhibit documented progression 10 months after starting treatment

[c] Patient met criteria for CR in terms of blood counts and clinical exam; however, bone marrow examination was declined and thus CR could not be confirmed

[d] Patient was refractory to fludarabine

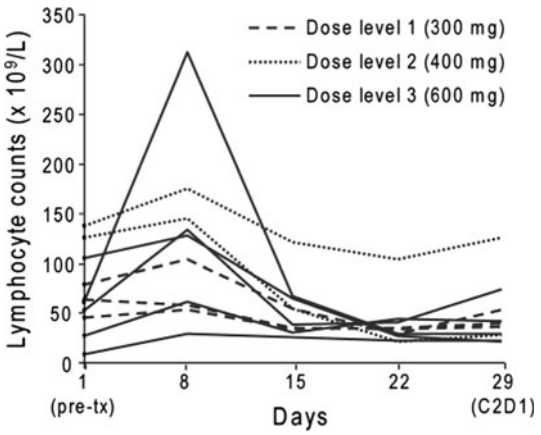

Fig. 1 Transient rise in lymphocyte count at day 8 of cycle 1. The plot represents the absolute lymphocyte counts for each patient (*y*-axis) prior to treatment (pre-tx) and then weekly until day 1 of cycle 2 (C2D1)

any study drug being given, in vitro cytotoxicity testing of patient lymphocytes revealed imatinib (5 or 10 μM) sensitization of CLL lymphocytes to chlorambucil in some but not all samples. However, on day 3, imatinib enhanced the sensitivity of *all* samples to chlorambucil, when compared to chlorambucil alone ($P < 0.0009$) (Fig. 3). Comparison of I values for chlorambucil in the presence of 5 or 10 μM imatinib on day 1, before treatment, and on day 3 revealed significant differences ($P = 0.009$, paired *t* test), indicating a greater degree of sensitization of CLL lymphocytes induced by 3 days of imatinib treatment. Moreover, there were statistically significant ($P < 0.03$) correlations between the IC_{50} of chlorambucil alone or with imatinib and the ratio of the lymphocyte count on day 15 when compared to pre-treatment lymphocyte counts (Fig. 4). As demonstrated in Fig. 4, the lower the IC_{50} value, the greater the decrease in lymphocyte count (i.e. the lower the ratio of day 15 to pre-treatment lymphocyte counts). Thus, the sensitivity of the lymphocytes to CLB alone or in combination with imatinib in vitro correlated with the effect of the drug combination in vivo on the patients' lymphocyte counts. The more sensitive the lymphocytes were to CLB or the combination, the greater the decrease in the lymphocyte count on day 15 when compared to day 1.

Discussion

The treatment rationale for this study was based on the hypothesis that imatinib sensitizes CLL lymphocytes to chlorambucil through inhibition of HR; this effect being mediated through inhibition of c-Abl by imatinib with downstream decreased activation of Rad51, a protein involved in repair of DNA damage. In support of this mechanism, our previous in vitro study showed decreased c-Abl phosphorylation, decreased Rad51 phosphorylation, and decreased nuclear foci of Rad51 in lymphocytes treated with imatinib and chlorambucil compared to chlorambucil alone [14]. Other in vitro studies showed that imatinib reduced Rad51 expression in two human glioma cell lines and sensitized the cells to the cytotoxic effects of ionizing radiation [15].

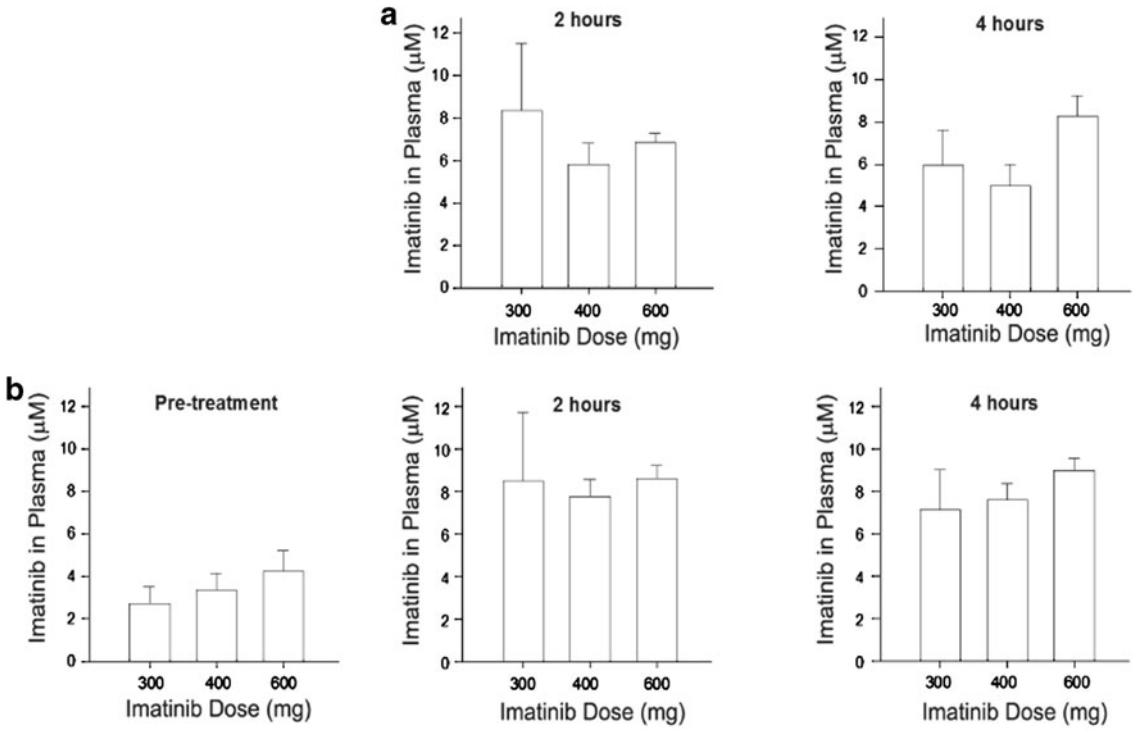

Fig. 2 Imatinib plasma concentration at cycle 1 **a** on day 1, at 2 and 4 h after imatinib administration, and **b** day 10, pre-treatment plus 2 and 4 h post-administration of imatinib. There was no significant difference in the plasma concentration of imatinib (*y*-axis) at the time points indicated, between the different doses levels of imatinib (*x*-axis)

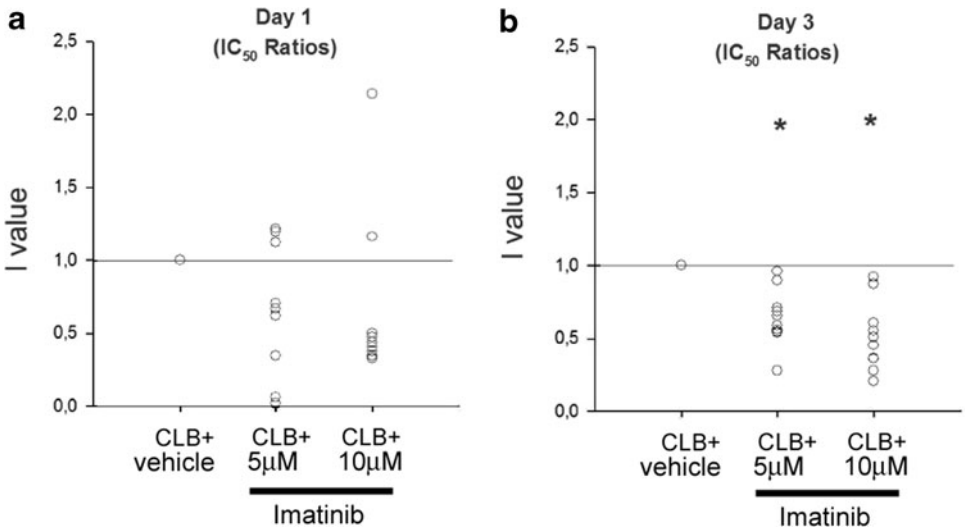

Fig. 3 The IC_{50} of chlorambucil (CLB) in the presence of vehicle, 5 or 10 μM imatinib was determined in vitro with lymphocytes isolated from patients on **a** day 1 (prior to treatment) and **b** day 3 (2 h after treatment with imatinib but prior to CLB). The sensitization values (*I* value) were obtained as described in the "Materials and methods" section. The I values were significantly <1 on day 3, thereby demonstrating synergy. The *asterisk* indicates a $P < 0.0001$

This was a phase I study of chlorambucil in combination with imatinib in adults with previously treated CLL. The prescribed regimen was generally well tolerated. Low-grade gastrointestinal toxicities (anorexia, diarrhea, nausea, and vomiting) were observed at all dose levels but were more prominent in patients receiving the highest dose of imatinib. Hematologic toxicity was minimal. One patient with stable disease (patient 2) had grade 2 thrombocytopenia and grade 3 neutropenia not requiring alteration of treatment schedule. This patient continued to have thrombocytopenia and neutropenia months after cessation of therapy, suggesting that it was disease related rather than due to treatment. At the highest dose level, we observed one dose-limiting toxicity (grade 3 thrombocytopenia), requiring cessation of therapy. This patient had grade 1 thrombocytopenia at baseline and continued to have grade 3 thrombocytopenia over one month after stopping treatment, again suggesting predominantly a disease-related phenomenon. Two patients had a serious adverse event (SAE) while on study. At the highest dose level, one patient (patient #7) experienced febrile

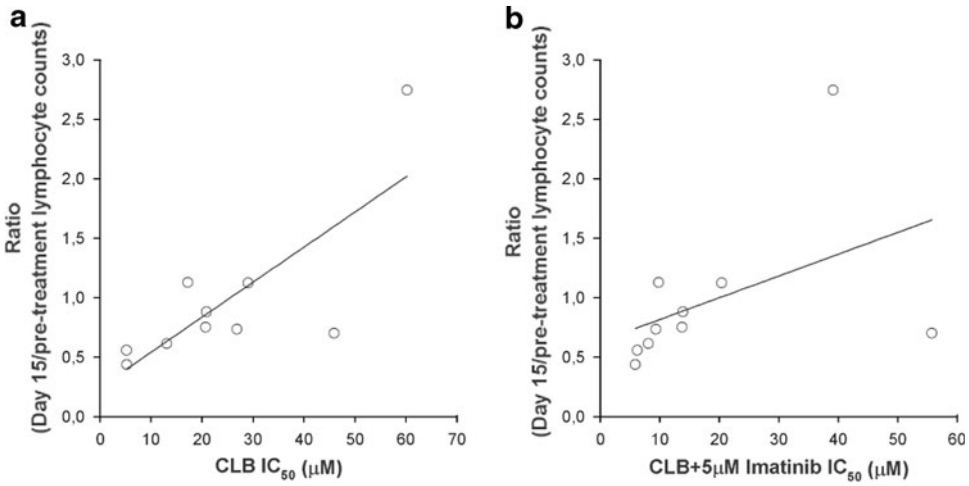

Fig. 4 Lymphocyte response expressed as the ratio of the day 15/pre-treatment lymphocyte counts (y-axis) correlates with **a** the IC_{50} of chlorambucil (CLB) alone and **b** the IC_{50} of CLB plus 5 μM imatinib (*x*-axis) obtained in vitro. The correlation coefficients were 0.67 and 0.77, respectively, with *P* values of 0.029 and 0.009

neutropenia during cycle 4, which was believed to be related to the treatment regimen. This patient was withdrawn and subsequently developed disseminated herpes. One patient with bulky lymphadenopathy at the 400 mg dose level (patient 5) experienced severe pneumonia, lung infiltration by CLL, and respiratory failure during cycle 2, which was believed to be disease related.

The combination of imatinib and chlorambucil shows promising evidence of efficacy in this pre-treated population, with five patients of 11 exhibiting a response, two of which were fludarabine refractory, and one of which had previously progressed on chlorambucil alone. In general, fludarabine refractory patients do not respond to chlorambucil [16]. There were two patients at dose level 3 with an unconfirmed CR since a bone marrow examination was declined.

Of the 11 patients enrolled in the study, only three patients completed the full 6 cycles, which limited the ability to make in vitro/in vivo correlations. In spite of this, in vitro results using patient lymphocytes isolated at day 1 and day 3 from our present study demonstrate that pre-treatment of patients with imatinib enhances sensitivity to chlorambucil in CLL. The mechanism of cell death in these lymphocytes appears to be through increased apoptosis [14].

Interestingly, most patients exhibited a pattern of transient exacerbation of lymphocytosis in the first 8 days, followed by progressively decreasing lymphocyte counts. The rapid and transient nature of the lymphocytosis suggests release of lymphocytes, perhaps from bone marrow and/or lymph nodes, rather than de novo lymphopoiesis. In fact, the patients with the greatest increases in lymphocyte counts on day 8 (>2-fold) all had bulky lymphadenopathy or massive splenomegaly. The effect of transient lymphocytosis in CLL in the context of decreasing lymphadenopathy was reported in a recent phase I trial of fostamatinib, an inhibitor of spleen tyrosine kinase (syk) [20].

It has been shown that c-Abl may act through the NF-κB pathway to prolong CLL lymphocyte survival and consequently, CLL cells with particularly high c-Abl expression are sensitive to apoptosis by treatment with imatinib [21]. However, the mean IC_{50}s of imatinib alone, as determined from patient lymphocytes isolated at day 1 (pre-treatment) and at day 3 (27 and 29 μM, respectively, data not shown), are not clinically attainable. Thus, it is unlikely that imatinib alone would be clinically useful in the majority of patients.

Given that the combination of chlorambucil and imatinib was well tolerated at the 400 mg dose level, and that the target serum levels of imatinib were achievable at all dose levels, we propose that 400 mg of imatinib given on days 1–10 and chlorambucil (8 mg/m^2 daily) on days 3–7 of a 28-day cycle, be used as the recommended phase II dose. This dose of imatinib represents the safest dose when considering the extensive experience in patients with CML [12]. Chlorambucil administered intermittently has a theoretical advantage over low-dose daily schedule in that it allows time between doses for the recovery of normal elements of the bone marrow, long before the regeneration of the more slowly proliferating leukemic lymphocytes [22]. That activity was seen with this regimen, and in particular, in two fludarabine refractory patients and in one patient who had progressed on chlorambucil alone, is an encouraging finding. Overall, the combination of chlorambucil and imatinib in patients with previously treated CLL was well tolerated and showed evidence of clinical efficacy. This regimen may be promising for the treatment of elderly patients with CLL in whom more aggressive, multiagent chemotherapy drugs are not suitable.

The concept of using tyrosine kinase inhibitors as synergistic therapy in conjunction with traditional chemotherapy is a promising and exciting new area applicable not only to CLL but possibly to other lymphoid and perhaps solid tumor malignancies as well. In support of this

concept, it has recently been demonstrated that short imatinib pulses added to chemotherapy in the treatment of Philadelphia-positive acute lymphoblastic leukemia (ALL) results in improvement in long-term survival [23]. Future studies, including a larger phase II study, will further define the efficacy and the mechanism of action of this regimen in CLL.

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