TITLE PAGE

Phase II Study of Dasatinib in Relapsed or Refractory Chronic Lymphocytic Leukemia

Short Title: Dasatinib in Relapsed or Refractory CLL

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STATEMENT REGARDING APPLICATION TO FUTURE PRACTICE OF CANCER MEDICINE:

This is the first completed clinical trial testing the effectiveness of dasatinib in chronic lymphocytic leukemia (CLL). We chose to test dasatinib in CLL because pharmacodynamic evaluation of this agent has demonstrated inhibition of Lyn kinase at very low nanomolar concentrations, and Lyn kinase has been shown to be a key enzyme in CLL survival. Recent in vitro studies have confirmed that dasatinib inhibits proliferation and triggers apoptosis in CLL cells. Our trial demonstrates responses according to strict NCI-WG criteria in 3 of 15 treated patients, which indicates effectiveness in this population of patients. We also report in vitro evidence that dasatinib inhibits phosphorylation of Lyn kinase with subsequent inhibition of Syk kinase associated with apoptosis at 6 hours, suggesting the mechanism of action. Future studies will determine the optimal dose and schedule of dasatinib in the treatment of CLL patients, either as a single agent or in combination.
ABSTRACT

Purpose:
CLL cells treated with dasatinib in vitro undergo apoptosis via inhibition of Lyn kinase. Thus, in this study we tested the activity of dasatinib in patients with relapsed CLL.

Experimental Design:
Patients were eligible for this phase 2 trial if they had documented CLL/SLL and had failed at least one prior therapy with a fludarabine-containing regimen and if they required therapy according to NCI-WG criteria. The starting dose of dasatinib was 140 mg daily.

Results:
Fifteen patients were enrolled, with a median age of 59 and a median of 3 prior regimens. All patients had received fludarabine, and 5 were fludarabine-refractory. Eleven of the 15 (73%) had high risk del(11q) or del(17p) cytogenetics. The primary toxicity was myelosuppression, with grade 3 or 4 neutropenia and thrombocytopenia in 10 and 6 patients, respectively. Partial responses by NCI-WG criteria were achieved in 3 of the 15 patients (20%; 90% CI 6-44). Among the remaining 12 patients, 5 had nodal responses by physical exam, and 1 patient had a nodal and lymphocyte response but with severe myelosuppression. Pharmacodynamic studies indicated apoptosis in peripheral blood CLL cells within 3-6 hours after dasatinib administration, associated with downregulation of syk mRNA.

Conclusions:
Dasatinib as a single agent has activity in relapsed and refractory CLL.
INTRODUCTION

B-cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the Western world and remains incurable outside of the bone marrow transplant setting.\(^{(1)}\) While the median survival for all newly diagnosed patients is over 10 years, there is significant patient to patient variability. For patients who relapse after initial treatment, survival varies from about 3 years in good risk patients treated aggressively to 9-13 months in fludarabine-refractory patients.\(^{(2-4)}\) The reasons for progression and treatment resistance are currently under intensive investigation.

CLL cells are believed to proliferate and escape apoptosis in large part from signal pathways originating from the B-cell receptor (BCR).\(^{(5)}\) Although the precise mechanisms likely differ between heavy chain mutated and unmutated CLLs, in both forms of the disease Src family kinases (SFKs) are overexpressed, are aberrantly located in the cytosol outside of lipid rafts, and appear to play a crucial role in promoting cell survival and proliferation.\(^{(6)}\) In unmutated CLL cells, the signaling from the BCR follows a pattern similar to that in normal B-cells. These cells respond to antigen engagement with translocation of the BCR to lipid rafts, where the immunoreceptor tyrosine-based activation motifs (ITAMs) located in the intracellular tails of the BCR become phosphorylated by the SFK Lyn.\(^{(7)}\) The BCR then phosphorylates Syk kinase, resulting in downstream activation of PI3K, Akt and Mcl-1 as well as MEK/ERK.\(^{(8, 9)}\) These events result in enhanced proliferation and survival. In mutated CLL cells, little response to BCR engagement is seen, and no migration of the BCR to lipid rafts is seen.\(^{(7)}\) Instead, these cells show constitutive activation of Lyn kinase outside lipid rafts in the cytosol with continual signaling of proliferation and survival pathways.\(^{(6)}\) In both mutated and unmutated CLL cells, inhibition of Lyn kinase by selective inhibitors enhanced apoptosis of these cells \textit{in vitro} through a caspase-dependent mechanism, further demonstrating the key role of Lyn in CLL.\(^{(6)}\)

Dasatinib is a tyrosine kinase inhibitor derived from an aminothiazole scaffold and was originally developed as a pan-Src kinase inhibitor. Once dasatinib was found to bind Abl with an affinity over 100 times that of imatinib mesylate, its development focused on CML, with successful clinical trials leading to its FDA approval in 2006 for the treatment of CML resistant to imatinib.\(^{(10, 11)}\) However, dasatinib also inhibits Lyn kinase \textit{in vitro} with an IC\textsubscript{50} of 11 nM\(^{(12)}\), similar to the 1-10 nM IC\textsubscript{50} of dasatinib for BCR-ABL.\(^{(10)}\) The inhibition of Lyn kinase by dasatinib has been tested in K562 cell lines resistant to imatinib mesylate, and in these cells, apoptosis correlated with inhibition of Lyn phosphorylation.\(^{(13)}\)

Dasatinib has been extensively studied in CML, initially at doses of 35 mg to 70 mg BID. Pharmacokinetic studies showed that the peak concentration of dasatinib occurred at approximately 2 hours while the half life was approximately 5 hours. Dasatinib was well tolerated.\(^{(14)}\) In a Phase I trial of dasatinib, CML cells from patients resistant to imatinib mesylate showed rapid and sustained inhibition of Lyn kinase.\(^{(14)}\) With the understanding that Lyn kinase may be critical for CLL survival, and given Lyn could be inhibited by dasatinib at tolerable doses, we initiated this phase 2 trial in patients with relapsed or refractory CLL. The use of 140 mg once daily was chosen due to emerging data that the once daily schedule was potentially less toxic and at least as effective in patients with CML.\(^{(15)}\)
MATERIALS AND METHODS

Trial Design

This was a single-arm, open-label, phase 2 study of dasatinib given once daily to patients with relapsed or refractory CLL/SLL. The study was undertaken from 2007 to 2008 at the Massachusetts General Hospital and Dana-Farber Cancer Institute, institutions of the Dana-Farber/Harvard Cancer Center. The primary objective of the study was to determine the overall response rate by NCI-WG criteria. Secondary objectives were to determine the duration of response, progression-free survival, overall survival, spectrum of toxicities, and pharmacodynamic studies to demonstrate Lyn kinase inhibition and caspase activation by dasatinib in peripheral blood CLL cells.

Patients

Eligible patients needed to have relapsed or refractory CLL or SLL, defined by flow cytometry or immunohistochemistry, with an immunophenotype positive for CD5, CD19, and CD23, with the exception that patients with CD23 negative cells could be eligible as long as cyclin D1 staining was also negative (ruling out mantle cell lymphoma). Patients needed to be at least 18 years of age, have failed at least 1 prior fludarabine containing regimen or at least 2 non-fludarabine containing regimens, have an ECOG performance status of 2 or better, creatinine <3.0 mg/dl, SGOT <3 x ULN (upper limit of normal), ANC >1,000/ul, platelets >50,000/ul, and reticulocyte count <10%. Patients were required to be in need of treatment according to NCI-WG guidelines. Women were excluded if pregnant, breast-feeding, or unwilling to use an acceptable form of contraception if of child-bearing potential. Additional exclusion criteria included uncontrolled angina, a prolonged QT interval at baseline (QTc >450 ms), clinically significant arrhythmias, significant hypertension, or known HIV infection. Drugs known to interfere with platelet function had to be stopped at least 7 days prior to starting dasatinib. The institutional review board of the participating institutions (Dana-Farber/Harvard Cancer Center IRB) approved this study and provided oversight for the duration of the trial. The data were analyzed primarily by P.C.A., E.C.A., D.N., L.W., and J.R.B, but all authors had access to the primary clinical trial data. The clinical trial registration number and name are NCT00438854 and “Dasatinib in Relapsed Chronic Lymphocytic Leukemia”. All patients were required to give written informed consent, and the trial was conducted in accordance with the Declaration of Helsinki.

Treatment

All patients began protocol treatment with a starting daily oral dose of dasatinib of 140 mg (a combination of two 20 mg and two 50 mg pills) and could continue treatment for up to 24 months if responding and without significant toxicity. Dasatinib was provided by Bristol-Myers Squibb. Treatment was intended to be continuous, with no planned rest period or breaks. Non-hematologic toxicities were graded by National Cancer Institute Common Toxicity Criteria (NCI CTC) version 3.0, and hematologic toxicities according to the 1996 NCI-WG guidelines. In the event of unacceptable toxicity, defined as any grade 2 or higher non-hematologic toxicity or any grade 3 or higher hematologic toxicity, the treatment was held until resolution of the toxicity.
to grade 1 or less. According to the degree of toxicity, drug could be re-instituted at the same
dose or with a dose reduction to 100 mg or 80 mg daily. If the 80 mg daily dose was not
tolerated, the patient was taken off protocol due to toxicity. Supportive measures such as blood
product support and growth factor administration were allowed at the discretion of the treating
physician, with no prophylactic anti-infective therapy mandated. Patients with progression of
disease were removed from study.

**Patient Evaluations**

Prior to study treatment, patients underwent the following baseline evaluations: history, physical
exam (nodes measured by ruler), complete blood count (CBC), chemistries, liver function tests,
human chorionic gonadotropin (HCG) in women, ECG, CT scans of the neck, chest, abdomen,
and pelvis, bone marrow biopsy with flow cytometry and cytogenetics with analysis by FISH for
del(11q), +12, del(13q), and del(17p). Expression of ZAP70 by CLL cells was determined by
flow cytometry or immunohistochemistry. In the first month of treatment, CBC, chemistries,
and liver function tests were performed at least weekly along with ECGs to assess the QTc
interval. For the first 6 months, similar laboratory studies were required at least monthly along
with an ECG for QTc assessment. At 2 month intervals up to the 6-month time-point, CT scans
were repeated to help assess their impact on response (though not required for NCI-WG criteria).
Also at the 2-month time point, a bone marrow biopsy was performed on all patients as a part of
response assessment. After 6 months, CT scans were required every 6 months. A bone marrow
biopsy was required to confirm a clinical CR or at the time of disease progression, but was not
required if patients were removed from the study due to other reasons such as toxicity.

**Pharmacokinetic Analyses**

For a subset of patients who consented to participate in the pharmacokinetic portion of the study,
plasma samples were obtained prior to the first dose of dasatinib and at 3, 6 and 24 hours after
dosing. Plasma levels of dasatinib were measured as previously described.(17)

**Peripheral blood lymphocyte enrichment**

Peripheral blood samples were obtained from patients at baseline, 3 hours, 6 hours, and 24 hours
after the first dose of dasatinib and prior to day 8 of therapy. Peripheral blood lymphocytes were
enriched by depleting non-lymphoid cells using the RosetteSep Human B Cell Enrichment
Cocktail (Stemcell Technologies, Vancouver, Canada).

**Apoptosis**

For analysis of apoptosis, samples of enriched peripheral blood lymphocytes (EPBLs) were
stained with anti-Caspase 3 antibody-FITC (Beckton Dickinson) and then fixed in
paraformaldehyde and ethanol. Tunel staining was performed using the APO-BRDU Tunel
Assay Kit (Invitrogen, Carlsbad, CA), except that anti-BRDU-Alexa Flouor 488 was replaced
with anti-BRDU-Alexa Fluor 647 (Invitrogen). Cells were analyzed using a FACSCalibur flow
cytometer (Beckton Dickinson).
**Elisa protein and phosphoprotein analyses**

For analysis of protein and phosphoprotein levels, EPBLs were lysed in RIPA lysis buffer with protease inhibitors (Sigma). The total protein concentration was identified using the BSA kit (Peirce). An enzyme-linked immunosorbent assay (ELISA) was used for detection of phosphoprotein levels. For p-Lyn, the human phospho-Lyn (Y397) DuoSet IC (R & D Systems, Minneapolis, MN) kit was utilized using 2 ug of total protein lysate according to the manufacturer’s instructions. For p-Syk, an ELISA plate was coated with rabbit anti-human p-Syk primary capture antibody (Cell Signaling) and the plate was blocked with BSA. A total of 2 ug of total protein sample lysate was incubated and the plate washed. A mouse anti-human Syk antibody (Sigma) was then added followed by goat anti-mouse biotin and, then streptavidin-linked horseradish peroxidase (both from R & D Systems). The plates were read colorimetrically and the amount of phosphoprotein was expressed as a ratio to the total protein concentration.

**Microarray Gene Expression Profiling**

RNA from EPBLs was extracted, processed, and applied to Affymetrix Human Plus 2.0 microarrays according to standard protocol. Nucleic acid quality was confirmed using gel electrophoresis.

**Statistical Analysis**

The primary objective of this phase II study was to determine the objective response rate (ORR) of patients with relapsed or refractory CLL/SLL treated with dasatinib. The primary measures of efficacy of tumor response were: complete remission (CR), nodular partial remission (nPR), or partial remission (PR), as per NCI-WG criteria. The true ORR is reported as percentage and 90% CI calculated using the binomial exact test. Patient clinical characteristics are summarized using numbers and percentages for categorical variables, median and range for continuous variables. Duration of on study time was defined from the date patients went on study to the date they were taken off study. Time to treatment failure (TTF) was defined from the date on study to date of progression, death in remission, initiation of non-protocol therapy in the absence of progression, or censored on the last visit if patients are still on study. Time to progression (TTP) was defined from the time of on study to the date of progression or censored on the off study date if patients had stopped the study. Patients still on study at the time of manuscript preparation were censored at the last visit date. OS was defined from the date on study to date of death or censored on the last visit if patients were still alive as the time of analysis. The method of Kaplan-Meier was used to summarize duration of on study, time to progression, time to treatment failure, and overall survival from the date of on study.

**Microarray Data Analysis**

In order to identify the differences between gene expression levels of pre and post treatment genes among patients 2 and 3, we first normalized the data at median, then specified the two comparison groups. The threshold of coefficient variation (standard deviation/mean) was set at 1.
and 1000 for lower and upper limit as filtering criteria and gene probes differentially expressed at a level of significance (p<.05) were identified.
RESULTS

Patients

Among the 15 patients enrolled between January 2007 and June 2008 (Table 1), there were 10 male and 5 female subjects with a median age of 59 years (40-78 years). Most were heavily pretreated with a median of 3 prior chemotherapy regimens and had advanced stage disease (7 with Rai stage 3 or 4). All patients had previously received fludarabine, and 5 patients were refractory. Pretreatment bone marrow biopsies demonstrated extensive infiltration (50-95% replacement) with CLL cells in 14 of the 15 patients. By cytogenetic/FISH analysis, there were 3 patients with del(17p), 5 patients with del(11q), and an additional patient with both del(17p) and del(11q). ZAP-70 expression was analyzed in all patients, and 10 patients were classified as positive with >50% expression, while the other 5 patients demonstrated low ZAP-70 expression.

Toxicities

All 15 treated patients were evaluable for toxicity, and hematological toxicities were frequently encountered (Table 2). Grade 3 and 4 neutropenia (ANC <1,000/ul) occurred in 10 patients. Grade 3 and 4 thrombocytopenia (NCI-WG criteria) occurred in 6 patients, but there were no bleeding events reported. Overall, myelosuppression was quite variable among the 15 patients, and 5 were removed from study within 4 months due to this toxicity rather than progression of disease. Two grade 3 infections were observed, both pneumonias that resolved with supportive care, and there were no grade 4 or 5 infections. The other most common non-hematologic toxicities encountered were nausea and fatigue, primarily grades 1 and 2 (Table 2). Four patients developed pleural effusions, which were transient and easily managed by holding the dasatinib and/or using diuretics and steroids. One patient had grade 4 cardiac toxicity due to an asymptomatic QTc of 516 ms, which resolved with repletion of potassium and magnesium. The one grade 4 renal toxicity was a serum potassium of 9.9 mmol/L that was spurious due to leukocytosis, as has been previously reported.(18) For 11 of the 15 patients, study drug was interrupted at some point due to toxicity, with a median duration of interruption of 1.5 weeks with a range of 0 to 8 weeks.

Response

Partial responses (PR) by NCI-WG criteria were achieved in 3 of the 15 patients (20%; 90% CI 6-44%). Among the 15 patients, 9 (60%) had nodal responses (2 CR and 7 PR) by physical exam (PE), 5 without a 50% reduction in lymphocytosis (see complete table provided as supplemental data). CT scans confirmed a 50% reduction of all nodal and extranodal masses in 4 of the 9 patients (Figure 1), with the other 5 showing less than 50% reduction. The 4 patients with 50% reduction in blood absolute lymphocyte count (ALC) also had at least 50% nodal responses, but 1 of these patients had severe myelosuppression such that the response could not be counted as a partial response by NCI-WG criteria. The blood responses tended to show both patterns of early and late improvement in lymphocytosis, with response within 7 days in one patient and ongoing improvement after 6 months in others (data not shown).

The median duration on study was 14 weeks. Ten patients came off study prior to 12 months, 3 of these with progression and 4 due to toxicity, while 3 withdrew consent without
progression or significant toxicity. At 12 months, 5 patients remained on study with one starting to progress but the other 4 continuing on treatment in response or with stable disease. The median TTF was 6.7 months. A total of 14 patients experienced treatment failure. Of the 14 patients, 10 of them had disease progression and 4 of them initiated new treatment without progression of disease. The median time to disease progression was 7.5 months. A total of 10 patients experienced disease progression and the rest were censored on the date off study (Figure 2). The median OS of all patients is 27 months. Of the 15 patients, 9 of them have died.

Analysis of ALC and lymph node responses showed that for the 5 patients with del(11q), 3 had a blood response and 5 had a nodal response. In fact, two of the three patients achieving NCI WG PR had del(11q). One of the 5 patients with del(17p) responded in lymph nodes but not in blood. Analysis of response by ZAP-70 status showed no clear pattern. Among the 10 ZAP-70 positive patients 1 responded in blood and 5 responded in lymph nodes. One of the 3 patients to respond according to NCI-WG responses was ZAP-70 positive.

Pharmacokinetics

The pharmacokinetics of dasatinib varied widely among the 3 patients tested, with the 3-hour level in plasma ranging from 19 to 112 ng/ml (see table provided as supplemental data). As expected given the five hour half-life of dasatinib, the 6-hour levels were far below the 3-hour levels, and the 24-hour trough levels were almost undetectable in all three patients.(14)

Pharmacodynamics

Using staining for TUNEL and activated caspase 3, apoptosis was seen in all three patients assessed, with maximal apoptosis 6 hours after dasatinib (Figure 3, and data not shown for caspase 3). While patient 1 had modest apoptosis, apoptosis was dramatic in 15-20% of enriched peripheral blood lymphocytes from patients 2 and 3.

Also within these three patients, we utilized Elisa to determine if levels of Lyn, p-Lyn, Syk, and p-Syk varied in the hours after taking dasatinib. Patients 1 and 3 had relatively low levels of p-Lyn relative to patient 2 prior to dasatinib (Figure 4). While levels of p-Lyn remained low in patients 1 and 3, the elevated p-Lyn in patient 2 decreased dramatically after dasatinib. Conversely, p-Syk levels, which were similar in all three patients prior to dosing, increased in patients 1 and 3 and slightly decreased in patient 2 following dasatinib. Of note, patient 2 had a partial response, while patients 1 and 3 did not experience clinical responses (see the associated ALC response at Day 8 provided at the bottom of Figure 4). Finally, the levels of Lyn kinase, p-Lyn, Syk, and p-Syk were compared at baseline and Day 8 of therapy. No association of baseline levels, or change between baseline and Day 8 levels, was seen with clinical response (data not shown).
Microarray results

In order to gain biologic insight into whether a dasatinib-induced change in transcriptional program was associated with the brisk apoptosis seen in patients 2 and 3, microarray gene expression profiling was conducted in EPBLs (enriched peripheral blood lymphocytes) and the expression of genes compared in samples obtained before and 6 hours after taking dasatinib.

We identified 123 probe sets, corresponding with 106 genes, which were differentially expressed. Of these, 102 probe sets were significantly downregulated and 16 probe sets were significantly upregulated in EPBLs 6 hours after taking dasatinib. Functional annotation analysis of genes was performed using the interface DAVID of the National Institutes of Health.

The 102 probe sets which were downregulated corresponded to 92 genes. Within the categories of lymphocyte activation/differentiation and leukocyte activation, the genes spleen tyrosine kinase (SYK), FK506 binding protein, early growth response 1 (EGR1), NLR family CARD 3, and Notch 2 were significantly (p < .05) downregulated (gene array provided as a supplemental figure).

The 16 upregulated probe sets corresponded to 14 unique genes. Among these, the genes GNAS1 and NFKB2 were significantly (p < .05) upregulated (gene array provided as a supplemental figure).
DISCUSSION

Here we report the first full study of dasatinib in CLL, although a case report has recently been published that shows a complete response in a patient treated with single-agent dasatinib.(19) This phase 2 study of single-agent dasatinib in patients with relapsed and refractory CLL demonstrated 3 partial responses by NCI-WG criteria among 15 patients treated. Overall 9 patients had shrinkage of lymph nodes or extranodal masses to less than 50% of their original size, and 4 patients had reduction of ALC by >50%. Dasatinib clearly has activity in relapsed CLL/SLL, and responses occurred in subgroups with poor prognostic indicators, particularly those with del(11q). Less activity was observed in patients with del(17p), and although 75% of patients were ZAP-70 positive, only 1 in 3 responders was ZAP-70 positive, suggesting that activity may be better in ZAP-70 negative patients. The small numbers in these subgroups make conclusions difficult, and subsequent studies with greater numbers of patients should reveal any true associations.

Dasatinib was reasonably well tolerated in this group of patients. The toxicity most frequently encountered was myelosuppression, with 10 patients experiencing grade 3 or 4 neutropenia. Severe thrombocytopenia occurred, but bleeding was not a problem, and both myelosuppression and thrombocytopenia resolved promptly after holding the drug. A lower dose of dasatinib or intermittent dosing might reduce the myelosuppression observed. Two patients did require treatment for grade 3 infections but these were easily managed and not unexpected given the patient population. The only other notable toxicities were nausea, pleural effusions, and fatigue. The nausea could be treated with anti-emetics, and the pleural effusions resolved with dose interruptions, diuretics, and steroids. The fatigue generally was mild and tolerable.

The greater activity of dasatinib in lymph nodes was not expected but welcome, since patients with advanced refractory disease often have significant lymphadenopathy and few treatment options. The reason for this increased activity in nodes is unclear but several possible explanations exist. Dasatinib may preferentially target proliferating CLL cells which are characteristically found in lymph nodes compared to blood.(20) Another possible explanation is that dasatinib interferes with stromal support of CLL cells in lymph nodes. The mechanisms of stromal support in CLL are complex and multifactorial. For example, it has been established that B-cell survival is enhanced by CD40 signaling.(21) CD40 is a transmembrane receptor that interacts with CD40 ligand present on CD4+ lymphocytes in lymph nodes, and this interaction results in the activation of PI3K and PLC\(\gamma\)2 followed by the up-regulation of NF-KB and other anti-apoptotic mediators such as survivin, Bcl-2 and Mcl-1.(20, 21) The intracellular mediator of CD40 signaling is Lyn kinase,(21) and it may be that the inhibition of Lyn by dasatinib interrupts this pathway of stromal support. More compelling is a report of an in vitro model which mimics nodal biology, where CD40 stimulation results in relative resistance to chemotherapy agents such as fludarabine through up-regulation in CLL cells of Bcl-xL, Mcl-1, and A1/Bfl-1, all anti-apoptotic proteins.(22) In this model, exposure to dasatinib completely prevented the entire anti-apoptotic program and rendered the CLL cells sensitive to fludarabine despite concurrent CD40 stimulation.(22) Hence, dasatinib inhibition of Lyn may be particularly important in lymph node biology, and may show greater activity as a chemotherapy sensitizer in combination therapy.

To date, the testing of dasatinib in combination with other drugs in CLL has only been performed in vitro, but these reports have been favorable. In one report, CLL cells from patients were treated in vitro with dasatinib, fludarabine, or in combination. Whereas modest apoptosis
was seen with each agent alone, combination treatment with both agents enhanced apoptosis by 50%. (11) Dasatinib led to global inhibition of tyrosine phosphorylation with down regulation of Akt, ERK1 or 2, MAPK, Bcl-xL, Mcl-1, and p38. (11) A similar result was demonstrated in a second report, which showed that CLL cells resistant to fludarabine or chlorambucil become sensitive to these agents when also exposed to dasatinib. (23) It seems apparent that, while dasatinib alone may not consistently initiate apoptosis in all CLL cells, the drug clearly inhibits survival pathways that become critical for CLL cells after attack by more conventional chemotherapy agents.

The current trial was originally based on the understanding that dasatinib, at concentrations achievable in humans, inhibits Lyn kinase which is overexpressed in CLL and mediates signal transduction from the BCR to downstream effector molecules. (6) To understand the activity of dasatinib further, we investigated levels of p-Lyn and p-Syk and the subsequent apoptosis of EPBLs in a subset of our patients. We were able to demonstrate among 3 patients studied, that one patient had constitutive Lyn phosphorylation that decreased after dasatinib, while the other two patients showed minimal change in levels of p-Lyn. In the patient in whom Lyn was inhibited, no p-Syk was seen and a clinical PR was observed. The other two patients, both of whom showed increased p-Syk following dasatinib dosing, did not show clinical responses. These results are consistent with a recent report that CLL cells exposed to dasatinib in vitro demonstrate widespread p-Lyn inhibition but undergo apoptosis only when p-Syk and PLCγ2 are also inhibited. (24) Interestingly, we identified changes in the transcriptional program of EPBLs associated with apoptosis after dasatinib exposure. Among the genes that underwent downregulation were SYK, which is implicated in lymphocyte signaling and survival and for which inhibitors are currently under clinical study in CLL. (25) FK506BP, which modulates the apoptotic signal of TGF-β in CLL, (26) EGR1, whose expression is associated with survival in CLL (27), NLR Card 3, which is downregulated 6 hrs following T-lymphocyte stimulation (28), and Notch 2, which similarly undergoes downregulation in CLL cells treated with proteasome inhibitors. (29) In contrast, genes which were upregulated included GNAS1, whose T393C polymorphism has been correlated with prognosis in CLL in one study (30) but not in another. (31) Also, NFKB2, which upregulates the anti-apoptotic bcl-2, was upregulated following exposure to dasatinib and may indicate the cell’s molecular attempt to evade apoptosis. (32) Our in vitro results can only be considered suggestive, and further studies need to be done to clarify the relationships between apoptosis and the various signaling molecules.

In conclusion, we have demonstrated in this study that dasatinib has single agent activity in relapsed and refractory CLL, particularly in lymph nodes and in high-risk del(11q) patients. Given this encouraging clinical data as well as reports of in vitro studies providing evidence for greater efficacy in combination with other agents, our future studies of dasatinib in CLL are focusing on combination therapy.
ACKNOWLEDGEMENTS

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AUTHORSHIP

Contributions: P.C.A. is the principal investigator, provided the original concept, and wrote the manuscript. E.C.A. assisted with the manuscript and contributed to the planning and conduct of the *in vitro* studies, which were performed with the help of K.M.L. J.R.B. provided overall guidance and oversight, and helped in the writing of the protocol and manuscript. Patient enrollment was provided by T.T., E.H., K.B., E.C.A., A.L., and J.R.B. All authors have reviewed this manuscript. The marrow samples with special staining were reviewed by R.H., and the statistical analysis was performed by L.W. and D.N.

Conflict-of-interest disclosure: This study is an investigator-initiated study supported by per patient funding from Bristol Myers Squibb, which also supplied dasatinib. P.C.A. received $1,500 for attending a Bristol-Myers Squibb Advisory Board Meeting regarding Hematologic Malignancies in 2007. J.R.B. served as a consultant for Calistoga, Celgene, and Genentech and received research funding from Celgene and Genzyme. The other authors have no conflict-of-interest to report.

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REFERENCES


### TABLE 1

**Patient Characteristics**

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<th>Total Number</th>
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<tr>
<td>Male/Female</td>
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<td>Age: median (range)</td>
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**Patients with Indicated Number of Prior Therapies (all had prior fludarabine):**

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<th>2 Prior</th>
<th>3 Prior</th>
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<td>5</td>
<td>2</td>
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**Prior Fludarabine-based Regimens Used (number of patients):**

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<th>F/Rituximab</th>
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<td>6</td>
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**Performance Status at Start of Treatment (number of patients):**

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<th>PS=2</th>
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**Median time from diagnosis to this treatment:** 7 years (range: 4-17 years)

**Baseline Hematologic Parameters:**

<table>
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</tr>
<tr>
<td>Median Hgb:</td>
<td>12.0 mg/dl (range: 9.0 mg/dl - 13.4 mg/dl)</td>
</tr>
<tr>
<td>Median Platelets:</td>
<td>112,000/ul (range: 58,000/ul - 228,000/ul)</td>
</tr>
</tbody>
</table>

**Baseline Rai Stage (number of patients):**

<table>
<thead>
<tr>
<th>Stage 0</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

**Baseline Bulky Nodes or Masses (>5 cm by Exam or CT Scan):** 7 patients

**FISH Cytogenetics (number of patients):**

<table>
<thead>
<tr>
<th>del(11q)</th>
<th>del(17p)</th>
<th>del(11q),del(13p),del(17p)</th>
<th>Tri(12) or del(13q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>
TABLE 2

Toxicities Encountered for the 15 Patients Treated*

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>None</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Fatal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heartburn</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pleural Effusion</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cardiac</td>
<td>12</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Renal</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>10</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>9</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Edema, wt gain</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Hematologic toxicities were graded according to NCI-WG criteria. The non-hematologic toxicities were graded according to the NCI CTC version 3.0. Values in table indicate number of patients for the grade listed.
FIGURE LEGENDS

Figure 1: Serial abdominal CT scans in patient A and patient B

Figure 2: A. Time to treatment failure among 15 patients with CLL/SLL treated with dasatinib
       B. Time to progression among 15 patients with CLL/SLL treated with dasatinib
       C. Overall survival among 15 patients with CLL/SLL treated with dasatinib

Figure 3: Apoptosis of CLL Cells from 3 Patients by Tunel Assay

Figure 4: Inhibition of Phosphorylation of Lyn Kinase and Syk Kinase in 3 Patients
FIGURE 2

A
Percentage of Patients on Treatment

Time to Treatment Failure in Months

B
Percentage of Patients Free from Progression

Time to Disease Progression in Months

C
Percentage of Patients Still Alive

Overall Survival in Months
FIGURE 3

Apoptosis of CLL Cells from 3 Patients by Tunel Assay

Blood was taken from patients before dosing, at 3 hr, and 24 hr after dosing with dasatinib.
FIGURE 4

p-Lyn and p-Syk in 3 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>p-Lyn 396:Total Protein</th>
<th>p-Syk:Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04 0.06 0.08 0.10</td>
<td>0.1 0.2 0.3 0.4 0.5</td>
</tr>
<tr>
<td>2</td>
<td>0.02 0.04 0.06 0.08 0.10</td>
<td>0.1 0.2 0.3 0.4 0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.02 0.04 0.06 0.08 0.10</td>
<td>0.1 0.2 0.3 0.4 0.5</td>
</tr>
</tbody>
</table>

ALC Responses in Blood in 3 Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>ALC at Baseline</th>
<th>ALC on Day 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16,000/ul</td>
<td>15,000/ul</td>
</tr>
<tr>
<td>2</td>
<td>131,000/ul</td>
<td>49,000/ul</td>
</tr>
<tr>
<td>3</td>
<td>84,000/ul</td>
<td>123,000/ul</td>
</tr>
</tbody>
</table>
# Clinical Cancer Research

## Phase II Study of Dasatinib in Relapsed or Refractory Chronic Lymphocytic Leukemia

Philip C. Amrein, Eyal C. Attar, Ronald W. Takvorian, et al.

*Clin Cancer Res* Published OnlineFirst March 14, 2011.

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<th>Updated version</th>
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</tr>
<tr>
<td>Author Manuscript</td>
<td>Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.</td>
</tr>
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