First report of phase II study of dasatinib with hyperCVAD for the frontline treatment of patients with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia

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Running title: HyperCVAD plus dasatinib in Ph+ ALL
ABSTRACT

Combination of cytotoxic chemotherapy and imatinib has improved the outcome for patients with Ph+ ALL. Dasatinib has significant clinical activity in patients with imatinib-resistance. We have examined the efficacy and safety of combining chemotherapy with dasatinib for patients with Ph+ ALL. Newly diagnosed patients receive dasatinib 50 mg po bid (or 100 mg daily) for the first 14 days of each of 8 cycles of alternating hyperCVAD and high dose cytarabine and methotrexate. Patients in complete remission (CR) receive maintenance daily dasatinib and monthly vincristine and prednisone for 2 years followed by dasatinib indefinitely. Thirty five patients with untreated Ph+ ALL with a median age of 53 years (range, 21 – 79) were treated; 33 (94%) patients achieved CR. Two patients died before response assessment from infections. Grade 3 and 4 adverse events have included hemorrhage and pleural and pericardial effusions. With a median follow up of 14 months (range 4-37), the median disease free survival and the median overall survival have not been reached with an estimated 2-year survival of 64%. Combination of chemotherapy with dasatinib is effective in achieving long term remissions in patients with newly diagnosed Ph+ ALL. This study was registered at www.ClinicalTrials.gov as NCT00390793.
INTRODUCTION

Prior to the introduction of tyrosine kinase inhibitors, the outcome of the majority of patients with Philadelphia-chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) was poor. Although complete remission (CR) could be achieved in most patients (60% - 90%), CR duration and disease-free survival (DFS) were short with few long term survivors. In older patients particularly those older than 60 years of age, the outcome was particularly dismal with high treatment-related mortality, low CR rates and low long term DFS and survival.\(^1\)

As a result, allogeneic stem cell transplantation (allo SCT) would be offered to all patients with a suitable donor in first CR. Of interest, even with these traditional regimens, the degree of reduction of BCR-ABL transcripts after induction and consolidation was a powerful predictor of disease response and survival.\(^2\)

However, the success of allo SCT has been limited due to its associated toxicity and the limited availability of donors. The introduction of tyrosine kinase inhibitors improved the likelihood of identifying a donor as these agents provide relatively durable responses allowing for the identification of a donor. In vitro studies demonstrated synergistic or additive effects against Ph+ cell lines when imatinib was combined with various cytotoxic agents, suggesting a potential role for these combinations in patients.\(^3\)-\(^5\) Several investigators explored the efficacy of imatinib in combination with chemotherapy for frontline treatment of patients with Ph+ ALL. Initially the optimal schedule was debated and concurrent as well as sequential schedules were investigated.\(^6\)-\(^11\)
In the first clinical trial reporting the combination of imatinib with chemotherapy, a CR rate of 96% with a 2-year disease-free survival of 85% was reported and half of the initial cohort of 20 underwent allo SCT. These were significantly superior to historical results using the chemotherapy regimen alone. Furthermore, molecular complete responses as analyzed by reverse transcription polymerase chain reaction (RT-PCR) were reported in 60% of patients. Importantly, there was no unexpected toxicity related to the addition of imatinib to the regimen. Other investigators have also reported the results of studies incorporating imatinib into chemotherapy regimens designed for ALL and the initial debates of concurrent versus sequential imatinib have been largely settled by several reports of improved efficacy and low toxicity with the concurrent regimens. In the early reports, the outcome of patients treated with such regimens was comparable whether the patients did or did not undergo an alloSCT in first CR, raising the debate of potential for “cure” without a transplant.

Both acquired and intrinsic resistance to imatinib has been described in patients with Ph+ ALL. Acquired imatinib resistance may be due to BCR-ABL-dependent mechanisms such as BCR-ABL overexpression or mutations in the kinase domains (KD). Other mechanisms of resistance independent of BCR-ABL have also been reported and include pharmacokinetic factors reducing the availability of imatinib within Ph+ cells, or activation of alternative signaling pathways such as the Src-kinase pathways.
Second generation inhibitors capable of overcoming such resistance are available. Dasatinib is a dual Src and Abl kinase inhibitor that binds both active and inactive moieties of the bcr-abl protein and is approximately 325 times more potent against the kinase in preclinical studies. The inhibition of Src may also be important in overcoming imatinib resistance particularly in lymphoid leukemias where Src kinase activity may be important in their pathogenesis. Dasatinib is active in vitro against all imatinib resistant BCR-ABL mutants with the exception of T315I. Significant activity of dasatinib in patients with Ph+ leukemias who were resistant to or intolerant of imatinib has been reported. Ottmann et al conducted a phase II study of dasatinib in 36 patients with Ph+ ALL after failing imatinib. The median age of the patients was 46 years (range, 15 to 85 years). Major hematological response was achieved in 15 (42%) of patients and cytogenetic CR in 21 (58%). They reported 6 patients with a baseline T315I mutation, none of whom responded. Response rates were similar in patients with other mutations compared to those with no mutations. Recently, administering dasatinib once daily in patients with Ph+ ALL produced similar responses and was associated with less toxicity with a lower incidence of myelosuppression or pleural effusions.

Based on the significant activity of dasatinib against BCR-ABL, and impressive data in patients with relapsed or imatinib-resistant disease, we hypothesized that the combination of dasatinib and chemotherapy would be effective in treating patients with Ph+ ALL and conducted this phase II study to examine the toxicity and efficacy of this regimen.
PATIENTS AND METHODS

Eligibility

Patients with previously untreated Ph+ ALL [determined by the identification of either t(9;22) karyotype and/or BCR-ABL fusion transcript] were eligible. Furthermore, patients had to be 18 years or older, have an Eastern Cooperative Group performance status of 2 or less, and have an adequate liver and renal function (with serum bilirubin ≤ 3.0 mg/dl, and a serum creatinine ≤ 3.0 mg/dl, unless considered due to tumor). Patients would be excluded if they had an active infection not controlled by antibiotics, clinical evidence of grade III-IV heart failure as defined by the New York Heart Association Criteria, active second malignancy, or prior history of treatment with dasatinib. Patients were also not eligible to participate if they were pregnant or breast feeding, had a history of bleeding diathesis, or a pleural or pericardial effusion thought not to be related to leukemia. All patients had to sign a consent form in accordance with the Declaration of Helsinki approved by the Institutional Review Board of the University of Texas – M D Anderson Cancer Center.

Treatment regimen

The details of the hyperCVAD regimen have been published previously.6,22 Odd courses (1, 3, 5, and 7) of hyperfractionated cyclophosphamide (Cytoxan), doxorubicin (Adriamycin), vincristine (Oncovin), and dexamethasone were given alternately with even courses (2, 4, 6, and 8) of high dose cytarabine and methotrexate. All even courses were preceded by a chest X-ray to ensure the
absence of a significant pleural effusion prior to the administration of methotrexate. Dasatinib 50 mg orally twice daily (or 100 mg orally daily following an amendment to the study when further data on the best dose and schedule of dasatinib became available\textsuperscript{21,23}) was administered in the first 14 days each of the above 8 courses. This was due to the concern for myelosuppressive property of dasatinib in particular in combination with intensive chemotherapy. For central nervous system (CNS) prophylaxis, intrathecal therapy with methotrexate and cytarabine was given alternately on days 2 and 7 of each course for a total of 6 or 8 doses depending on the risk of CNS relapse (based on serum lactate dehydrogenase and the marrow proliferative index). For patients presenting with active CNS disease, confirmed by cytological examination of the cerebrospinal fluid (CSF), the above regimen was repeated twice weekly until the CSF became clear of leukemic cells and the CSF cell count normalized; then the patients received intrathecal therapy once weekly for 4 weeks, or until the initiation of the next cycle of chemotherapy when the above stated prophylactic regimen was resumed. Cranial irradiation was not administered for prophylaxis, but patients presenting with or developing cranial nerve palsies would receive radiation to the base of the skull in addition to intrathecal therapy.

Maintenance therapy was given for 2 years with monthly courses of intravenous vincristine and 5 days of oral prednisone 200 mg daily and was initiated following the completion of the 8 courses of chemotherapy (or earlier due to poor tolerability and toxicity). Dasatinib 50 mg orally twice daily (or 100 mg daily) was administered throughout the planned 2 year maintenance and was
continued after that indefinitely. We omitted the antimetabolites 6-mercaptopurine and methotrexate in order to avoid compromising the dose of dasatinib, felt to be the most effective agent to prevent relapse. Maintenance therapy could be interrupted on months 6 and 13 with intensification courses of hyperCVAD and dasatinib. Patients with no evidence of minimal residual disease (MRD) who were deemed poor candidates for such intensification continued maintenance uninterrupted. Appropriate dose reductions for the cytotoxic agents according to the type and degree of side effects and according to previously published measures were permitted.6,22 For dasatinib, both during initial therapy and the maintenance period, dose reductions to 70 mg or 50 mg orally daily were allowed for significant drug-related toxicity and dose escalation to 140 mg daily was permitted for inadequate response. At any time, during the intensive or maintenance phases, patients with an available matched donor had the option to proceed to an allogeneic stem cell transplant.

**Supportive care**

Supportive care measures were implemented according to standard guidelines. Tumor lysis prophylaxis with allopurinol or alternatives such as rasburicase, as well appropriate intravenous hydration and alkalinaztion was administered in the first course to all patients. Prophylactic antibiotic therapy with oral levofloxacin or trimethoprim/sulfamethoxazole, valacyclovir, fluconazole, or equivalent alternatives were provided to all patients during the periods of neutropenia. Transfusion of blood, platelets or other blood products were used
according to established guidelines to support periods of cytopenia or coagulopathy.

**Follow-up assessments**

All patients underwent baseline evaluation including history and physical examination, complete blood count with differential, full chemistry including renal and hepatic panel, bone marrow aspirate for histology, flow cytometry, cytogenetic, fluorescent in situ hybridization (FISH) and reverse transcription quantitative polymerase chain reaction (RQ-PCR) for the *BCR-ABL* transcripts and DNA PCR for immunoglobulin heavy chain gene rearrangements.

Bone marrow evaluations were repeated approximately on days 14 and 21 of the first cycle of treatment. Complete blood counts, electrolytes, and renal and hepatic indices were performed at least weekly during the intensive cycles of chemotherapy. Bone marrow aspiration material was assessed by morphology, cytogenetics, flow cytometry and *BCR-ABL* RQ-PCR every 2-3 cycles.

Cerebrospinal fluid assessment was performed at baseline on day 2 of induction chemotherapy at the time of administration of the first intrathecal chemotherapy. Baseline cardiac function was evaluated using a multigated radionuclide ventriculography (MUGA) scan or an echocardiogram and this assessment was repeated if clinically indicated.

**Minimal residual disease monitoring techniques**

**Molecular monitoring**

*BCR-ABL* RQ-PCR was performed on total RNA extracted from leukocytes following red blood cell lysis. Reverse transcription was performed
using random hexamers and PCR performed using TaqMan primer/probes for the e1a2, e13a2 (b2a2), and e14a2 (b3a2) BCR-ABL transcripts in a single tube with normalization to total ABL transcripts. Post-PCR capillary electrophoresis was used to type splice form, with the method having a sensitivity of approximately 1 in 10,000 BCR-ABL expressing cells as established by periodic dilution studies.\textsuperscript{24} Major molecular response (MMR) was defined as a BCR-ABL/ABL ratio of less than 0.05%. BCR-ABL kinase domain (KD) mutation analysis covering codons 221 to 500 was performed on cDNA using a nested PCR strategy.\textsuperscript{25} For cases containing T315I mutation, quantitation of mutation levels was performed using a pyrosequencing-based strategy with a sensitivity of detection of 1%.\textsuperscript{25}

\textit{IGH} clonality studies were performed on extracted genomic DNA using separate FR1, FR2 and FR3 PCR reactions with a consensus J primer. The sensitivity of detection of this method in a sample with low numbers of polyclonal B cells (such as post-treatment BM and CSF) is approximately 0.2-1%.

\textit{Multi-parameter flow cytometry}

Minimal residual disease assessment by flow cytometry was performed on whole bone marrow specimens using a standard stain-lyse-wash procedure. 1 × 10\textsuperscript{6} cells were stained per analysis tube, and data were acquired on 2 × 10\textsuperscript{5} cells when specimen quality permitted. In the initial part of the study, data on four-color staining combinations were acquired on FACSCalibur cytometers using CellQuest software (BD Biosciences, San Diego, CA) and analyzed using FlowJo (TreeStar, Ashland, OR). Starting in 3/2009, data on six-color stains were
acquired on FACSCanto cytometers using FACSDiva software (BD Biosciences) and analyzed using FCS Express (De Novo Software, Los Angeles, CA). Four-color combinations contained CD19-PerCP-Cy5.5 and CD34-APC in all tubes, with additional antigens conjugated to FITC and PE including CD10, CD13, CD15, CD20, CD22, CD25, CD33, CD38, CD45, CD58, CD66c, and CD81 (all antibodies from BD except CD10 from Beckman Coulter, Fullerton CA and CD66c from Immunotech, Marseilles, France). Six-color combinations included CD34-PerCP-Cy5.5, CD10-PE-Cy7, and CD19-APC-H7 in each tube, with the additional antigens listed above conjugated to FITC, PE and APC. MRD was identified in comparison with the known patterns of antigen expression by normal maturing CD19+ B cells, using an approach similar to that described by Weir et al. A distinct cluster of at least 20 cells showing altered antigen expression was regarded as an aberrant population, yielding a sensitivity for both four-color and six-color assays of 1 in 10,000 cells (for adequate specimens where $2 \times 10^5$ cells could be collected). We required aberrant expression of at least 2 antigens to make a diagnosis of MRD.

**Response and outcome definitions**

Complete response (CR) was defined as the presence of less than 5% blasts in the bone marrow (BM) with more than $1 \times 10^9$/L neutrophils and more than $100 \times 10^9$/L platelets in the peripheral blood (PB) with no extramedullary disease. Relapse was defined by recurrence of more than 5% blasts in a BM aspirate unrelated to recovery or by the presence of extramedullary disease. CR duration (CRD) was calculated from the time of CR until relapse. Disease-free
survival (DFS) was calculated from the time of CR until relapse or death from any cause. Event-free survival (EFS) was calculated from the beginning of treatment until an event including relapse, death during induction or death in CR. Overall survival (OS) was calculated from the time of diagnosis until death.

**Statistical methods**

Survival curves were plotted by the Kaplan-Meier method and compared using the log-rank test. Differences in subgroups by different covariates were evaluated using the chi-square test for nominal values, and the Mann-Whitney U and Fischer’s exact test for continuous variables.

**RESULTS**

**Patients and treatment**

From 9/28/2006 to 7/15/2009, 35 patients with untreated Ph+ ALL have been enrolled in the study and treated. The study has completed accrual and this is the first report of the patients’ outcome. The pre-treatment characteristics of the patients are given in table 1. Their median age at presentation was 53 years (range, 21 – 79); 20 (57%) patients were older than 50 years and 11 (31%) older than 60 years. Their median white blood cell (WBC) count at diagnosis was 17.4 x 10^9/L (range, 1.7-284.3 x 10^9/L). Four patients (11%) had central nervous system (CNS) involvement at presentation. Twenty four patients (69%) had the e1a2 transcript, 5 (14%) the e13a2, 3 (9%) both the e13a2 and e14a2, 1 (3%) the e14a2, and 1 (3%) the e1a3 transcript; and 1 (3%) patient in which the
breakpoint was not detected by the RQ-PCR assay but had a *BCR-ABL* fusion by FISH.

Overall these 35 patients have received a total of 187 courses of therapy and the median number of courses per patient is 6 (range, 1 to 8). So far, 23 patients have received maintenance therapy and 4 have completed all 24 courses of maintenance.

**Response to induction**

All but 2 patients are evaluable for assessment of response to induction (Table 2). Thirty-three (94%) achieved CR after first course; two patients (6%) died before response assessment from infections; in both patients, bone marrow exam on day 14 showed no detectable disease. The median time to achieving CR was 23 days (range, 16 to 43).

Among the patients who achieved CR, 27 (82%) achieved cytogenetic (CG) CR after one course of therapy; 4 (12%) had persistent Ph+ metaphases (3 had 5% and one 15%), and 2 (6%) had insufficient metaphases (IM). Overall, 31 (94%) patients eventually achieved CG CR. One patient remained persistently Ph+, and a second patient’s last bone marrow exam prior to death had IM. Of the 33 patients who achieved CR, 20 (61%) achieved complete molecular remission (CMR) at a median of 14 weeks (range, 2 – 59) and 7 additional (21%) achieved MMR at a median of 11 weeks (range, 2-51). MRD assessment by flow cytometry is negative in 29 of 33 (88%) patients at a median of 3 weeks (range, 2-18 weeks) Figure 1 shows the levels of residual disease after one cycle of
protocol therapy in CR for the entire cohort, as measured by $BCR-ABL/ABL$ RQ-PCR or by flow cytometry.

**Follow-up and outcome**

Figures 2a and 2b demonstrate the MRD status by PCR and by flow cytometry with follow-up. All patients remaining in CR became negative for minimal residual leukemia by flow cytometry but some had persistent low level $BCR-ABL$ transcripts. With a median follow up of 14 months (range, 4 – 37 months), 26 patients are alive and 24 are in CR with an estimated 2-year survival of 64% (95% Confidence Interval, CI; 38%-81%) and event-free survival (EFS) of 57% (95% CI; 34%-74%). Figures 3a, b, c and d demonstrate the EFS, CR duration, disease free survival and overall survival of the patients. Four patients died in CR; 1 from an unrelated cardiac event and 3 from infections. Five patients have relapsed with a median response duration of 57 weeks (range, 32 - 72) and 3 of them have died. Two of the relapsed patients were non-compliant with Dasatinib during maintenance. Two relapsed patients are alive after receiving further chemotherapy in the form of hyperCVAD with nilotinib followed by nilotinib or imatinib maintenance. Neither underwent an allogeneic stem cell transplant and both are in complete morphologic and cytogenetic remission with a major molecular response. Three of 4 patients with detectable minimal residual leukemia by flow cytometry at 3 months or beyond eventually relapsed. Furthermore, recurrence of a positive flow cytometry value from a previous negative predicted for relapse. In 4 patients morphological relapse was
preceded by flow and molecular relapse (Figure 2). Three of 5 patients with relapse had ABL mutations (1 T315I, 1 F359V, and 1 V299L); no mutations were detected in 1 other patients and 1 patient was not tested. Overall, only 4 patients have undergone an allogeneic stem cell transplant in first CR (3 with MMR and 1 with no molecular response prior to transplant) and all are alive and disease-free following transplant. At any time, during the intensive and maintenance phases, patients with an available donor had the option to proceed to an allogeneic stem cell transplant. Many patients, however, chose to continue on the study despite the iteration that transplant in first CR continues to be the standard of care.

Three patients were taken off study for reasons other than death, transplant, or relapse. Two were taken off due to toxicity and commenced on Imatinib. In one patient the leukemic blasts were CD 20+ and rituximab was added to his therapy.

**Toxicity**

The median time to neutrophil and platelet recovery for cycle 1 was 18 and 23 days. The median time to platelet and neutrophil recovery for subsequent courses was 20 and 15 days (ranges, 0-37 and 0-35 days, respectively). Toxicity has included 16 episodes of bleeding (11 GI, 2 GU, 1 soft tissue hematoma and 2 subdural hematomas), and 8 episodes of pleural effusions. These include 2 episodes in the induction cycle and 6 in the subsequent cycles in 8 different patients; 6 episodes were of grade I/II severity and 2 of grade III/IV severity (Table 3). Other adverse events included infections, deep vein thromboses and pulmonary emboli, diarrhea, metabolic abnormalities including
hypophosphatemia, hypokalemia, hypocalcemia, hyperglycemia, elevated transaminases, and reversible rise in creatinine unrelated to treatment. Adverse events during the study are summarized in table 3.

During induction and consolidation courses, all patients received dasatinib at the prescribed 100 mg daily; 2 patients received a reduced dose of dasatinib (70 mg daily) for 3 days while having an infection. At maintenance, 22 patients started dasatinib at 100mg daily and one at 50 mg daily. Of the 22, 14 remained on 100mg daily, and 7 were dose reduced (4 to 70mg daily, 2 to 50mg daily, and 1 to 50mg qod), one patient’s dose was increased to 140mg daily due to an increasing PCR value for BCR-ABL. The reasons for dose reduction were cytopenias, pleural effusions, diarrhea, elevation of ALT, pericardial effusion, worsening renal function, and infections.

DISCUSSION

Several recent reports have better defined the outcome of patients with Ph+ ALL who underwent an allogeneic stem cell transplant in the pre-imatinib era.28,29 Fielding et al reported that only 28% of patients in their study actually underwent allogeneic stem cell transplant as designed by the protocol.28 Therefore, although durable remissions are possible with allogeneic stem cell transplantation performed in first CR, alternative strategies are needed for the majority of patients with this disease who are not candidates for or unable to undergo transplant. Furthermore, initial studies combining imatinib and chemotherapy have clearly established the feasibility and efficacy of this strategy
with some of the early reports suggesting a possible advantage for patients who were not transplanted in first CR. However, with further follow-up, late relapses and death from toxicity occur and the initial advantage may not be maintained.

Mutations in the kinase domain of BCR-ABL have been reported to be an important mechanism of resistance to imatinib and their role in primary resistance and relapse in patients with ALL receiving the imatinib containing regimens is becoming clearer. Whether these mutations exist prior to the initiation of therapy or develop as a result of treatment-related clonal selection remains unclear. Early studies of patients with advanced Ph+ lymphoid leukemias identified KD mutations in most patients with acquired imatinib resistance. However, KD mutations occurring prior to initiation of therapy with imatinib and accounting for primary resistance were not identified. More recently, using more sensitive techniques, the presence of low-level KD mutations in imatinib-naïve patients have been reported. The frequency of the mutant allele at the time of diagnosis was always below the level of detection by direct cDNA sequencing and ATP-binding P-loop mutations were the dominant type accounting for 83% of the mutations with the other 17% being T315I. Remarkably, pre-existence of mutations including T315I did not adversely affect the CR rate or the achievement of molecular CR when compared with patients who only had unmutated BCR-ABL at diagnosis. However, other investigators were unable to detect such pre-existing mutations in their cohort. It is clear, however, that such
mutations occur commonly in relapse. This was further confirmed in the current study where the majority of patients in relapse had mutations, including T315I.

Dasatinib is a more potent inhibitor of the tyrosine kinase activity of BCR-ABL in vitro and overcomes the majority of BCR-ABL resistance mutations.\textsuperscript{17} Furthermore, phase II trials have demonstrated significant activity of dasatinib in patients with Ph+ ALL who have failed prior imatinib.\textsuperscript{20} We, therefore, hypothesized that addition of dasatinib to chemotherapy may be more effective in maintaining responses than imatinib. Due to the concern for myelosuppression, we opted to give dasatinib 50 mg twice daily and only for 14 days each cycle to allow for the recovery of blood counts. With the release of data on enhanced safety and equivalent efficacy of once daily dosing, the protocol was amended and all subsequent patients received a once daily dose of 100 mg with chemotherapy and during maintenance.\textsuperscript{21,35}

Overall, the regimen was well-tolerated although grade 3 and 4 side effects including several bleeding episodes as well as pleural effusions requiring treatment with steroids or discontinuation of therapy did occur. Dasatinib has a reported deleterious effect on platelet function which is likely to be exacerbated by chemotherapy-induced thrombocytopenia.\textsuperscript{36,37} Furthermore, we avoided the use of proton pump inhibitors during the administration of dasatinib for the concern of drug interactions. The incidence of other adverse events such as pleural effusions was not significantly higher than that reported in single agent studies of dasatinib in Ph+ ALL and advanced phase CML.\textsuperscript{38} Furthermore, the combination of chemotherapy and dasatinib did not result in unacceptable
myelossuppression with the median time to platelet and neutrophil recovery for the induction course being 23 and 18 days, respectively (ranges, 18-44 and 14-22 days, respectively). The regimen was better tolerated after achieving CR with rapid recovery of blood counts and fewer grade 3 and 4 adverse events in the subsequent courses of therapy. The length of follow-up for the majority of patients on the study is limited; therefore, the extent of toxicities and particularly any toxicity related to prolonged therapy with dasatinib is not yet clear.

With a median follow-up of 14 months (range 4-37 months), 26 (74%) patients are alive and 24 (69%) are leukemia free. This compares favorably with historical data using the hyperCVAD regimen without a tyrosine kinase inhibitor and is similar to our previous regimen of hyperCVAD plus imatinib (p=NS).30 In this study, only 4 patients (10%) underwent an allogeneic stem cell transplant in first CR as compared with 16 of 53 (20%) of patients in the imatinib study. Despite this, the outcomes appear comparable in the two trials. Furthermore, survival and EFS at 2 to 3 years are of similar magnitude as was reported for the MRC/ECOG trial and the City of Hope with patients undergoing transplantation in first CR (Figures 4a and b).

The degree of reduction of disease burden at CR and with follow-up as measured by QT-PCR was superior for the dasatinib containing regimen than our historical cohorts (data not shown). Previous reports have suggested that achievement of molecular responses in patients with Ph+ ALL is associated with a superior outcome.2 Therefore, using a regimen that is consistently associated with reduction of disease burden at remission and with follow-up may be
associated with an improved outcome. It has been previously shown that a higher total number of days of tyrosine kinase inhibitor administration in induction and consolidation may be associated with an improved outcome. We administered dasatinib for only 14 days per each cycle of chemotherapy and due to infections and toxicity significant delays between periods of dasatinib treatment was possible. Future studies where dasatinib is administered continuously throughout consolidation will demonstrate whether this strategy can improve the outcome further. Similarly, other strategies such as the administration of monoclonal antibodies like rituximab as well as risk-adapted therapy based on the level of MRD are being pursued in an attempt to reduce relapse and toxicity, and improve survival.

Recently, the Gruppo Italiano Malattie Ematologiche dell’ Adulto (GIMEMA) has reported the results of the LAL1205 study using dasatinib with steroids and intrathecal chemotherapy for the frontline treatment of adult patients (median age 54 years, range, 24-76) with Ph+ ALL. A CR rate of 100% after 57 days of therapy with an overall survival of 81% at 10 months was reported. There were no induction deaths and only 2 patients discontinued therapy due to toxicity. At the time of report, 9 of 35 patients enrolled had relapsed after a median of 72 days after the end of induction. No data was provided on the post-remission therapy received. The authors concluded that the use of chemotherapy for induction of older patients with Ph+ ALL is questionable. We evaluated the outcome of patients enrolled on this study by age, and as expected, the overall survival was superior for younger patients (Figure 4c and d). It is arguable that
older patients may benefit from a less intensive regimen but the ideal strategy remains to be defined.

In conclusion, we have demonstrated the feasibility of combining chemotherapy with dasatinib in patients with Ph+ ALL. The regimen is effective in achieving long-term leukemia free survival even without an allogeneic stem cell transplant in first CR. Other strategies including continuous dosing of dasatinib or the addition of monoclonal antibodies may help further improve the outcome.
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AUTHORSHIP AND CONFLICT OF INTEREST STATEMENT

Farhad Ravandi, Designed and conducted the trial, provided patients, analyzed data. Wrote the manuscript

Susan O’Brien, Deborah Thomas, Stefan Faderl, Dan Jones, Jeffrey Jorgensen, Partow Kebriaei, Richard Champlin, Gautam Borthakur, Jan Burger, Alessandra Ferrajoli, Guillermo Garcia-Manero, William Wierda, Jorge Cortes, Provided patients and material, reviewed the manuscript

Rebecca Garris, Analyzed the data, reviewed manuscript

Samuel Dara, Collected the data

Hagop Kantarjian, Designed the trial, provided patients, reviewed the manuscript

Farhad Ravandi, Received research support, has been member of advisory boards and has received honoraria from Bristol-Myers Squibb

Jorge Cortes, Hagop Kantarjian, Received research funding from Bristol-Myers Squibb

Susan O’Brien, Deborah Thomas, Stefan Faderl, Dan Jones, Jeffrey Jorgensen, Partow Kebriaei, Richard Champlin, Gautam Borthakur, Jan Burger, Alessandra Ferrajoli, Guillermo Garcia-Manero, William Wierda, Rebecca Garris, Samuel Dara, no conflict of interest
REFERENCES:


**Table 1 - Pre-treatment patient characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Previously untreated</th>
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<tr>
<td>Patients, N</td>
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<tr>
<td>Median age,(years) [Range]</td>
<td>53 [21-79]</td>
</tr>
<tr>
<td>Median WBC (x 10^9/L) [Range]</td>
<td>17.4 [1.7-284.3]</td>
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<td>PS</td>
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<td>0-1</td>
<td>33 (94)</td>
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<td>2</td>
<td>2 (6)</td>
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<tr>
<td>CNS disease at start</td>
<td>4 (11)</td>
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<td>Cytogenetics</td>
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<td>9 (25)</td>
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<td>Ph+, +other</td>
<td>24 (69)</td>
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<tr>
<td>CD20 expression ≥20%</td>
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**Legends** – WBC: white blood cell count; IM: insufficient metaphases; ND: not done
Table 2 – Responses assessed after induction cycle

<table>
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<th>Response</th>
<th>N=35 (%)</th>
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<tr>
<td>Complete hematological response (CHR)</td>
<td>33 (94)</td>
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<td>Early death (ED)</td>
<td>2 (6)</td>
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<td>Complete cytogenetic response (CG CR)</td>
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<tr>
<td>Undetectable by flow cytometry</td>
<td>17 (52)</td>
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<tr>
<td>Complete molecular response (CMR)</td>
<td>7 (21)</td>
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<td>Major molecular response (other than complete)(MMR)</td>
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</tbody>
</table>
**Table 3** – Treatment related toxicities encountered during induction and consolidation intensive chemotherapy cycles.

<table>
<thead>
<tr>
<th></th>
<th>Induction Number (%) n=35</th>
<th>Subsequent cycles Number (%) n=31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 1/2</td>
<td>Grade 3/4</td>
</tr>
<tr>
<td>Infections</td>
<td>1 (3) 24 (69)</td>
<td>1 (3) 26 (84)</td>
</tr>
<tr>
<td>Pleural effusions</td>
<td>1 (3) 1 (3)</td>
<td>5 (16) 1 (3)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>- 4 (11)</td>
<td>1 (3) 11 (35)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>14 (40) 1 (3)</td>
<td>3 (10) -</td>
</tr>
<tr>
<td>Cardiac</td>
<td>3 (9) 1 (3)</td>
<td>2 (6) 1 (3)</td>
</tr>
<tr>
<td>Renal Failure</td>
<td>- 6 (17)</td>
<td>- 4 (13)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>22 (63) 21 (60)</td>
<td>9 (29) 11 (35)</td>
</tr>
<tr>
<td>▲ AST/ALT</td>
<td>4 (11) 3 (9)</td>
<td>2 (6) 1 (3)</td>
</tr>
<tr>
<td>Neurological</td>
<td>2 (6) -</td>
<td>2 (6) 1 (3)</td>
</tr>
<tr>
<td>DVT/PE</td>
<td>1 (3) -</td>
<td>3 (10) 4 (13)</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

Figure 1. Minimal residual disease after 1 cycle at CR by a) BCR-ABL/ABL% and b) flow Cytometry

Figure 2. a) Minimal residual disease by time from therapy by BCR-ABL/ABL%. The orange line connects the median values of the patients at the stated time-points. Several patients at different time intervals had overlapping values. In one patient BCR-ABL was undetectable at presentation by RQ PCR and was detected by FISH b) Minimal residual disease by time from therapy by multi-parameter flow cytometry

Figure 3. a) Event-free survival, b) Complete remission duration, c) Disease-free survival, d) Overall survival. Numbers of patients at risk are indicated on the horizontal axis

Figure 4. a) Complete remission duration excluding patients who underwent allogeneic stem cell transplant in first CR b) Overall survival excluding patients transplanted in first CR c) Complete remission duration by age d) overall survival by age. Numbers of patients at risk are indicated on the horizontal axis.
**Figure 1.** Level of residual disease assessed after 1 cycle at CR by a) RQ-PCR for BCR-ABL transcripts or b) flow cytometry

<table>
<thead>
<tr>
<th>MRD by BCR-ABL/ABL% (n=32/33)</th>
<th>% patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;=1</td>
<td>31</td>
</tr>
<tr>
<td>&lt;=1; &gt;.05</td>
<td>25</td>
</tr>
<tr>
<td>MMR</td>
<td>22</td>
</tr>
<tr>
<td>CMR</td>
<td>22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MRD by Flow Cytometry (n=28/33)</th>
<th>% patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pos</td>
<td>39</td>
</tr>
<tr>
<td>Neg</td>
<td>61</td>
</tr>
</tbody>
</table>
**Figure 2a.** Minimal residual disease by time from therapy by BCR-ABL/ABL%
Figure 2b. Minimal residual disease by time from therapy by flow cytometry
Figure 3a. Event-free survival

Event Free Survival Probability

Total Fail
35 11

2 yr EFS = 57%

Months from Start of Treatment

0 12 24 36
n=18 n=8 n=2
Figure 3b. Complete remission duration

Complete Remission Duration Probability

<table>
<thead>
<tr>
<th>Months from Response Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>12 (n=17)</td>
</tr>
<tr>
<td>24 (n=8)</td>
</tr>
<tr>
<td>36 (n=2)</td>
</tr>
</tbody>
</table>

Total Fail
33 5
2 yr CRD = 70%
Figure 3c. Disease-free survival

Total    Fail
33       9

2 yr DFS = 60%
Figure 3d. Overall survival

Total  Fail
35  9

2 yr OS = 64%
Figure 4a. Complete remission duration excluding patients undergoing allogeneic stem cell transplant in first CR

- Total: 29
- Fail: 5

2 yr CRD = 67%

Complete Remission Duration Probability

Months from Response Date
Figure 4b. Overall survival excluding patients transplanted in first CR

2 yr OS = 61%

Total  Fail
31    9

Overall Survival Probability

Months from Start of Treatment

n=17  n=8  n=2
Figure 4c. Complete remission duration by age

CRD

Complete Remission Duration Probability

Total Fail

<60 23 3
>=60 10 2

Months from Response Date
Figure 4d. Overall survival by age

Overall Survival by age

Overall Survival Probability

Months from Start of Treatment

Total Fail

<60 24 5

>=60 11 4

n=13, n=5  n=6, n=3  n=1, n=1
First report of phase II study of dasatinib with hyperCVAD for the frontline treatment of patients with Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia

Farhad Ravandi, Susan O'Brien, Deborah Thomas, Stefan Faderl, Dan Jones, Rebecca Garris, Samuel Dara, Jeffrey Jorgensen, Partow Kebriaei, Richard Champlin, Gautam Borthakur, Jan Burger, Alessandra Ferrajoli, Guillermo Garcia-Manero, William Wierda, Jorge Cortes and Hagop Kantarjian