Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a single-centre, phase 2 study

Elias Jabbour, Hagop Kantarjian, Farhad Ravandi, Deborah Thomas, Xuelin Huang, Stefan Faderl, Naveen Pemmaraju, Naval Daver, Guillermo Garcia-Manero, Koji Sasaki, Jorge Cortes, Rebecca Garris, C Cameron Yin, Joseph D Khoury, Jeffrey Jorgensen, Zeev Estrov, Zachary Bohannan, Marina Konopleva, Tapan Kadia, Nitin Jain, Courtney DiNardo, William Wierda, Vicky Jeanis, Susan O'Brien

Summary

Background Combination of chemotherapy with a tyrosine-kinase inhibitor is effective in the treatment of Philadelphia chromosome-positive acute lymphoblastic leukaemia. Ponatinib is a more potent BCR-ABL1 inhibitor than all other tyrosine-kinase inhibitors and selectively suppresses the resistant T315I clones. We examined the activity and safety of combining chemotherapy with ponatinib for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia in this continuing phase 2 trial.

Methods In this single-centre, phase 2, single-arm trial, adult patients with previously untreated Philadelphia chromosome-positive acute lymphoblastic leukaemia were sequentially enrolled. Patients who had received fewer than two courses of previous chemotherapy with or without tyrosine-kinase inhibitors were also eligible. Patients had to be aged 18 years or older, have an Eastern Cooperative Oncology Group performance status of 2 or less, have normal cardiac function (defined by ejection fraction above 50%), and have adequate organ function (serum bilirubin ≤3·0 mg/dL and serum creatinine ≤3·0 mg/dL, unless higher concentrations were believed to be due to a tumour). Patients received eight cycles of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) alternating with high-dose methotrexate and cytarabine every 21 days. Ponatinib 45 mg was given daily for the first 14 days of cycle 1 then continuously for the subsequent cycles. Patients in complete remission received maintenance with ponatinib 45 mg daily with vincristine and prednisone monthly for 2 years followed by ponatinib indefinitely. The primary endpoint for this study was event-free survival. The trial is registered at ClinicalTrials.gov, number NCT01424982.

Findings 37 patients were enrolled and treated from Nov 1, 2011, to Sept 1, 2013. 2-year event-free survival rate was 81% (95% CI 64–90). Grade 3 or more toxic effects included infections during induction (20 [54%] patients), increased aspartate aminotransferase and alanine aminotransferase concentration (14 [38%] patients), thrombotic events (three [8%]), myocardial infarction (three [8%]), hypertension (six [16%]), skin rash (eight [22%]), and pancreatitis (six [16%] patients). Two patients died from from myocardial infarction potentially related to treatment; another patient also died from myocardial infarction related to sepsis. Two further patients died, one from bleeding and another from infection, both deemed unrelated to treatment.

Interpretation The first results of this ongoing trial indicate that the combination of chemotherapy with ponatinib is effective in achieving early sustained remissions in patients with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukaemia. New strategies, including dosing titration of ponatinib and optimised control of vascular risk factors, might further improve outcomes.

Funding ARIAD Pharmaceuticals Inc.

Lancet Oncol 2015; 16: 1547–55

Published Online
September 30, 2015
http://dx.doi.org/10.1016/S1470-2045(15)00207-7

See Comment page 1451

Department of Leukemia (E Jabbour MD, Prof H Kantarjian MD, Prof F Ravandi MD, Prof D Thomas MD, Prof S Faderl MD, Prof N Pemmaraju MD, N Daver MD, Prof G Garcia-Manero MD, K Sasaki MD, Prof J Cortes MD, R Garris, Prof Z Estrov MD, Z Bohannan MA, M Konopleva MD, T Kadia MD, N Jain MD, C DiNardo MD, Prof W Wierda MD, V Jeanis RN, Prof S O'Brien MD), **Department of Biostatistics** (Prof X Huang PhD, Prof J Jorgensen MD), **and Department of Hematopathology** (C C Yin MD, J D Khoury MD), **The University of Texas MD Anderson Cancer Center, Houston, TX, USA**

Correspondence to:
Dr Elias Jabbour, Department of Leukemia, The University of Texas, MD Anderson Cancer Center, 1515 Holcombe Blvd, Box 428, Houston, TX 77030, USA
ejabbour@mdanderson.org

Introduction

Incorporation of BCR-ABL1 tyrosine-kinase inhibitors with chemotherapy has substantially improved the survival outcomes of patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia.[1–3] The combination of cytotoxic chemotherapy with tyrosine-kinase inhibitor is now the standard of care for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia. It has been established that tyrosine-kinase inhibitor treatment should be started immediately upon recognition of Philadelphia chromosome-positive disease, and that continuous exposure to them is superior to pulsed or intermittent administration.[4–9]

Despite the high efficacy of this combination, the 3-year event-free survival and overall survival rates of patients with Philadelphia chromosome-positive adult acute lymphoblastic leukaemia are only about 40% and 60%, respectively.[1,2] This low survival can mostly be attributed to resistance to tyrosine-kinase inhibitors. Both acquired and intrinsic resistance to tyrosine-kinase inhibitors have been described.[10,11] Acquired resistance might be due to BCR-ABL-dependent mechanisms such as BCR-ABL overexpression or mutations in the kinase domains; many patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia relapse with a T315I clone, which is resistant to imatinib and

Research in context

Evidence before this study

Tyrosine-kinase inhibitors in combination with chemotherapy are one of the primary therapies for Philadelphia chromosome-positive acute lymphoblastic leukaemia. Unfortunately, many malignancies acquire resistance to most tyrosine-kinase inhibitors through the T315I mutation in the BCR-ABL1 fusion protein. We sought to address this important major clinical issue by combining chemotherapy with a new tyrosine-kinase inhibitor, ponatinib, that can effectively target both wild-type and T315I BCR-ABL1.

Added value of this study

We show that the combination of ponatinib with the hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) chemotherapy regimen results in durable responses in Philadelphia chromosome-positive acute lymphoblastic leukaemia. Lower doses of ponatinib might be needed due to toxic effects, and a potential strategy is to start patients on ponatinib and then transition to another kinase inhibitor once response is attained.

Implications of all the available evidence

Ponatinib in combination with hyper-CVAD represents an effective treatment for Philadelphia chromosome-positive acute lymphoblastic leukaemia, with high proportion of patients achieving durable response. Further refinement of the combination might result in new standards of care for these patients.

second-generation tyrosine-kinase inhibitors.[12] The rates of T315I mutation depend on the regimen used but range from 33% to 70% in patients who relapse after being treated with dasatinib-based regimens.[2,13,14] These high mutation rates and the intractability of T315I-mutant clones suggest an urgent need for new tyrosine-kinase inhibitors that can target T315I-mutant Philadelphia chromosome-positive acute lymphoblastic leukaemia.

Ponatinib is the most potent BCR-ABL1 inhibitor with activity recorded in leukaemias with both wild-type and mutated BCR-ABL1, including T315I.[15] Clinical trials of ponatinib have shown its high activity in Philadelphia chromosome-positive chronic myeloid leukaemia and Philadelphia chromosome-positive acute lymphoblastic leukaemia; the proportion of patients achieving complete cytogenetic response is 50–70% in patients failing to respond to previous treatment with two to three tyrosine kinase inhibitors and in those with T315I mutated BCR-ABL1.[16,17]

We hypothesised that the combination of chemotherapy and ponatinib might be associated with a higher proportion of patients achieving a response, fewer patients developing resistance, and a higher likelihood of eradication of minimal residual disease than that reported with a combination of other tyrosine kinase inhibitors and chemotherapy. We report the first results of an ongoing phase 2 study to assess the efficacy and safety of this combination.

Methods

Study design and participants

Adult patients with previously untreated Philadelphia chromosome-positive acute lymphoblastic leukaemia, established by the identification of either t(9;22) karyotype or BCR-ABL1 fusion transcript, were eligible and enrolled sequentially at the University of Texas MD Anderson Cancer Center (Houston, TX, USA). Patients who had received two or fewer courses of previous chemotherapy with or without tyrosine-kinase inhibitors were also eligible. Patients had to be aged 18 years or older, have an Eastern Cooperative Oncology Group performance status of 2 or less, have normal cardiac function (defined by ejection fraction above 50%), and have adequate organ function (serum bilirubin ≤3·0 mg/dL and serum creatinine ≤3·0 mg/dL, unless higher concentrations were believed to be due to a tumour). Patients were excluded if they had an active infection not controlled by antibiotics, clinical evidence of grade 3 to 4 heart failure as defined by the New York Heart Association criteria,[18] active second malignancy, or previous history of treatment with ponatinib.

All patients underwent baseline assessment, which included history and physical examination; complete blood count with differential; full chemistry panel (including renal and hepatic panel); bone marrow aspiration for histology, flow cytometry, cytogenetics, fluorescent in situ hybridisation, and reverse-transcription quantitative polymerase chain reaction (RT-qPCR) for BCR-ABL1 transcripts; and DNA PCR for immunoglobulin heavy-chain (IGH) gene rearrangements. BCR-ABL1 kinase domain (KD) mutational testing was not done at baseline.

All patients were enrolled consecutively and signed a consent form in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center. There were no competing trials at our institution.

Procedures

The hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) regimen was 300 mg/m^2 cyclophosphamide intravenously over 2–3 h every 12 h for six doses on days 1–3, with sodium mercaptoethanesulfonate at twice the total dose as cyclophosphamide but given by continuous infusion starting with cyclophosphamide and ending 12 h after the last dose; 2 mg of vincristine intravenously on days 4 and 11; 50 mg/m^2 of doxorubicin intravenously over 2 h on day 4; and 40 mg of dexamethasone daily on days 1–4 and days

11–14. Patients received eight cycles of hyper-CVAD alternating with high-dose methotrexate and cytarabine every 21 days.[19,20] Ponatinib was given at 45 mg orally daily for the first 14 days of cycle 1 then continuously for the next seven cycles of consolidation chemotherapy (eight cycles in total). Rituximab 375 mg/m^2 iv was also given during the first four cycles in patients with CD20 expression of 20% or more of blast cells.[21] Flow cytometry was performed on diagnostic bone marrow aspirates to establish lineage and CD20 expression. Bone marrow aspirates collected at the time of morphological complete response were assessed for minimal residual disease when feasible. For CNS prophylaxis, intrathecal therapy with methotrexate and cytarabine was given alternately on days 2 and 7 of each cycle for a total of 12 doses. For patients presenting with active CNS disease, confirmed by cytological examination of the CSF at baseline, the intrathecal regimen was repeated twice weekly until the CSF became clear of leukaemic cells and the CSF cell count normalised. After normalisation, these patients received intrathecal therapy once per week for 4 weeks or until initiation of the next cycle of chemotherapy, when the regimen was resumed. Cranial irradiation was not given for prophylaxis, but patients presenting with or developing cranial nerve palsies received radiation to the base of the skull in addition to intrathecal therapy.

Following completion of eight cycles of hyper-CVAD and ponatinib, maintenance therapy of daily oral ponatinib 45 mg was given for 2 years with monthly (30-day) courses of intravenous vincristine on day 1 and oral prednisone 200 mg daily on days 1–5. Initiation of maintenance due to treatment-related toxic effects before completion of eight cycles was implemented if appropriate. Months 6 and 13 of maintenance were designed as intensification courses of hyper-CVAD and ponatinib, the same as induction. Patients with no evidence of minimal residual disease who were poor candidates for such intensification continued maintenance therapy uninterrupted. Daily oral ponatinib at a dosage of 45 mg was continued indefinitely after the maintenanace therapy.

Appropriate dose reductions for the cytotoxic drugs according to the type and degree of side-effects or toxic effect were permitted and followed previously published guidelines.[19,20] Ponatinib dose reductions to 30 mg or 15 mg orally daily were allowed for significant drug-related toxic effects during both initial therapy and the maintenance period. Beginning on Aug 1, 2014, the protocol was amended due to concerns over potential vascular toxicity of dasatinib; ponatinib would be given at 45 mg daily for 14 days during induction therapy and then at 30 mg daily continuously starting with the second cycle, which could be further reduced to 15 mg daily continuously once a complete molecular response was achieved. At any time during the intensive or maintenance therapy phases, patients with an available matched donor had the option to proceed to an allogeneic stem-cell transplant. The decision to proceed with an allogeneic stem-cell transplant was at the discretion of the treating physician and previous experience at our institution.[22]

Supportive care measures were implemented according to standard guidelines. Tumour lysis prophylaxis with allopurinol, or alternatives such as rasburicase, and appropriate intravenous hydration were given in the first course to all patients. Prophylactic antimicrobial therapy, including oral levofloxacin or trimethoprim-sulfamethoxazole, antiviral prophylaxis, and antifungal prophylaxis with azoles or echinocandins, was given to all patients during periods of neutropenia beginning in induction. Transfusions of blood, platelets, or other blood products were given according to established guidelines to support periods of cytopenia or coagulopathy.

Bone marrow assessments were repeated at around days 14 and 21 of the first cycle of treatment. Complete blood counts, electrolytes, and renal and hepatic indices were obtained at least weekly during the intensive cycles of chemotherapy. Bone marrow aspiration material was assessed by morphology, cytogenetics, flow cytometry, and BCR-ABL1 RT-qPCR every two to three cycles.

CSF was assessed on day 2 of induction chemotherapy at the time of administration of the first intrathecal chemotherapy. Baseline cardiac function was assessed with a multigated radionuclide ventriculography scan or transthoracic echocardiogram, and this assessment was repeated if clinically indicated.

BCR-ABL1 RT-qPCR was done on total RNA extracted from leucocytes after red blood cell lysis. Reverse transcription was done with random hexamers, and PCR was done with TaqMan primers and probes for the e1a2, e1a3, e13a2 (b2a2), and e14a2 (b3a2) BCR-ABL1 transcripts in a single tube with normalisation to total ABL transcripts. Post-PCR capillary electrophoresis was used to determine the type of fusion transcripts, with the method having a sensitivity of about 1 in 10 000 BCR-ABL1-expressing cells, as established by periodic dilution studies.[23] Major molecular response was defined as a BCR-ABL1/ABL1 ratio of less than 0·1% (international scale).[24] Complete molecular response was defined as undetectable disease at or below the 10^{-5} level. BCR-ABL1 KD mutation analysis that covered codons 221–500 was done on cDNA with a nested PCR strategy at the time of treatment failure (defined by lack or loss of response).[23] For patients with a T315I mutation, quantitation of mutation concentrations was done with a pyrosequencing-based strategy with a detection rate of 1%.[25]

IGH clonality studies were done on extracted genomic DNA with 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 (post-treatment bone marrow and CSF) is about 0·2–1%.

Assessment of minimal residual disease by multiparameter flow cytometry was done on whole bone marrow specimens by using a standard stain–lyse–wash

procedure using six-colour combinations of CD34, CD10, and CD19, as previously described.[25] A distinct cluster of at least 20 cells that showed altered antigen expression was regarded as an aberrant population, which yielded a sensitivity of 1 in 10 000 cells (for adequate specimens in which 2×10^5 cells could be collected).

Outcomes

The primary outcome was improvement in the 3-year event-free survival, calculated from the beginning of treatment until an event (relapse, death during induction, or death during complete remission) occurred. The secondary outcomes were proportion of patients achieving an overall response, duration of response, overall survival, calculated from the time of treatment initiation until death, and safety. Complete remission was defined as the presence of fewer than 5% blasts in the bone marrow, with more than 1×10^9 cells per L neutrophils and more than 100×10^9 per L platelets in the peripheral blood and no extramedullary disease. Relapse was defined by recurrence of more than 5% lymphoblasts in a bone marrow aspirate unrelated to recovery or by the presence of extramedullary disease, and all patients underwent BCR-ABL1 mutation testing at time of relapse. Duration of complete remission was calculated from the time of complete remission until relapse.

Statistical analysis

This report is a first analysis of an ongoing study originally designed to accrue 60 patients and powered to assess improvement in event-free survival as the primary endpoint. The initial study design was based on our previous experience with hyper-CVAD plus imatinib or dasatinib, for which the reported median event-free survival was 24 months. The current study has 94% power to prove if the combination of hyper-CVAD and ponatinib can achieve an improvement in median event-free survival to 36 months. The trial was continuously monitored, with an early stopping rule in place if it was ever likely that the trial's event-free survival was less than that of previous similar trials. No stopping rules were met. The event-free survival analysis and all other statistical analyses are descriptive.

Survival curves were plotted by the Kaplan-Meier method and compared with the log-rank test. Differences in subgroups by different covariates were assessed with the χ^2 test for nominal values and the Mann-Whitney *U* and Fisher exact tests for continuous variables. Significance was defined as a p value less than 0·05. The trial is registered at ClinicalTrials.gov, number NCT01424982.

Role of the funding source

The funder provided free study drug and funding for a research nurse for this study. The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From Nov 1, 2011, to Sept 1, 2013, the first 37 patients were consecutively enrolled and treated in this ongoing study. 34 (92%) patients had untreated Philadelphia chromosome-positive acute lymphoblastic leukaemia and three (8%) had been previously treated; two patients had active disease status after one previous cycle of chemotherapy without tyrosine kinase inhibitor before Philadelphia chromosome-positive and BCR-ABL1 status was identified, and one patient had complete cytogenetic response after two cycles of chemotherapy and dasatinib. 11 (30%) patients were CD20-positive and received rituximab during the first four cycles. 18 (49%) patients had no baseline cardiovascular risk factors. Patient characteristics are summarised in table 1.

Overall, patients received a median of six cycles (range 2–8 cycles) of therapy. As of September, 2015, 13 patients are receiving maintenance therapy and are in complete remission. Nine patients were given an allogeneic stem cell transplant after a median of four treatment cycles.

All patients were assessable for response (table 2). Negative minimal residual disease assessed by six-colour multiparameter flow cytometry was recorded in all but one patient (97%). Early mortality (death within 4 weeks of treatment start) did not occur.

Of the 32 patients with Philadelphia chromosome-positive metaphases at the start of therapy, complete cytogenetic response, as assessed by conventional cytogenetics, was achieved in 30 (94%) patients after one course of induction therapy. One patient with a minor cytogenetic response (95% Philadelphia chromosome-positive metaphases) after induction therapy converted to complete response after a second course. Furthermore, molecular response was achieved in most patients: major molecular response was achieved in 35 (95%) of 37 patients overall and in 24 (69%) of those 35 patients after eight cycles of induction therapy. The median time to major molecular response was 3 weeks (range 2–14; one cycle) and to complete molecular response 11 weeks (range 2–96; about three cycles). The median time to minimal residual disease negativity was 3 weeks (range 3–14). Figure 1 shows the levels of residual disease after one cycle of therapy in complete remission for the entire cohort as measured by BCR-ABL1/ABL1 RT-qPCR or by flow cytometry. Figure 2 shows minimal residual disease status by PCR and by flow cytometry with follow-up.

With a median follow-up of 26 months (range 15–39), 29 (78%) patients were in complete remission, with nine (24%) patients receiving allogeneic stem-cell transplantation, for an estimated 2-year survival of 80% (95% CI 63–90), 2-year complete remission duration of 97% (80–99·6), and 2-year event-free survival of 81% (64–90).

	Participants (n=37)
Age	
Median (years)	51 (27–75)
≥50 years	20 (54%)
≥60 years	12 (32%)
Males	20 (54%)
ECOG performance status	
0–1	31 (84%)
2	6 (16%)
White blood cells (×10⁹ per L)	8 (1–630)
CNS disease	3 (8%)
CD20-positive	11 (30%)
BCR-ABL1 transcript	
p190	27 (73%)
p210	10 (27%)
Cytogenetics	
Diploid	5 (14%)
Philadelphia chromosome-positive	32 (86%)
Baseline cardiovascular risk factors	
Hypertension	18 (49%)
Dyslipidaemia	4 (11%)
Coronary artery disease	4 (11%)
Peripheral arterial disease	1 (3%)

Data are n (%) or median (range). ECOG=Eastern Cooperative Oncology Group.

Table 1: **Patient characteristics**

	Number of patients (%)
Complete response*	36/36 (100%)
Complete cytogenetic response†	32/32 (100%)
Major molecular response	35/37 (95%)
Complete molecular response	29/37 (78%)
Flow cytometry negative‡	35/36 (97%)

Data are n/N (%). *One patient in complete response at beginning of study. †Five patients were diploid by conventional cytogenetics at beginning of study. ‡One patient had no sample sent to flow cytometry.

Table 2: **Best overall response**

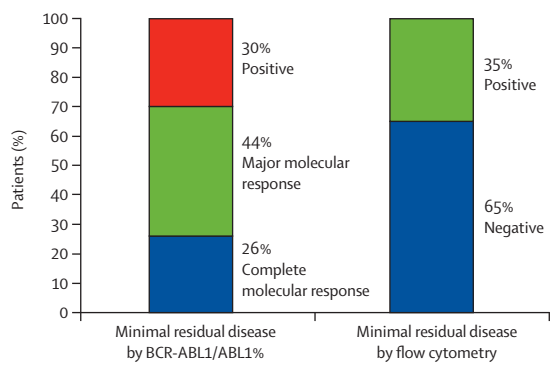

Figure 1: **Levels of residual disease after one cycle of protocol therapy in complete response**
Minimal residual disease after one cycle at complete remission by BCR-ABL1/ABL1 percentage and flow cytometry.

Figure 3 shows the overall survival and event-free survival of the patients.

Six patients died in complete remission: the first from an unrelated cardiac event 4 months after electing to discontinue therapy because of deconditioning and logistical reasons, after being placed on imatinib; the second from multiple organ failure after sepsis in neutropenia after the second cycle (day 13); the third from a head injury sustained after a fall after cycle 4 (day 13); and the fourth from sepsis and multiple organ failure after allogeneic stem-cell transplantation. The two other patients had no risk factors and died of arterial vascular events early in the study that might have been the result of the high initial ponatininb doses. The first patient was aged 37 years, was receiving ponatinib 45 mg daily, and had a non-ST elevation myocardial infarction after cycle 2 (day 41). The second was a woman aged 54 years, was receiving ponatinib 30 mg daily, and had unexplained chest pain on day 42 of cycle 4 that preceded her death. Two (5%) patients relapsed after a median of 18 months (range 10–26). The first patient had achieved complete molecular response and then decided to switch to dasatinib because of concerns about the vascular events encountered in ponatinib trials; she relapsed 6 months after changing treatment. No kinase domain mutation was found in this patient. She failed to respond to two salvage regimens and died. The second patient achieved a complete molecular response for 23 months and was on maintenance therapy with ponatinib at 15 mg daily. She relapsed with no kinase domain mutation identified and achieved a second complete remission after blinatumomab and dasatinib therapy.

Overall, nine patients underwent allogeneic stem-cell transplantation when in first complete remission (seven with major molecular response and two with complete molecular response before transplantation). One patient died from complications of transplantation and the others are alive and disease-free after transplantation. There was no difference in overall survival when patients were censored or not at the time of allogeneic stem-cell transplantation (figure 4). After transplantation and engraftment, tyrosine-kinase therapy was resumed (two patients on imatinib, three on dasatinib, one on nilotinib, and two on ponatinib).

Median time to platelet recovery was 22 days (range 17–35) and median time to neutrophil recovery was 18 days (13–29) for cycle 1, and 22 days (0–35) and 16 days (0–28), respectively, for all eight cycles. Adverse events are presented in table 3. Venous thrombotic events were recorded in three patients, with one renal vein thrombosis and two pulmonary emboli. These events occurred early on during the induction phase, did not recur, and did not further affect ponatinib therapy. Three patients died from myocardial infarction, of which two were unexplained and one was in the context of

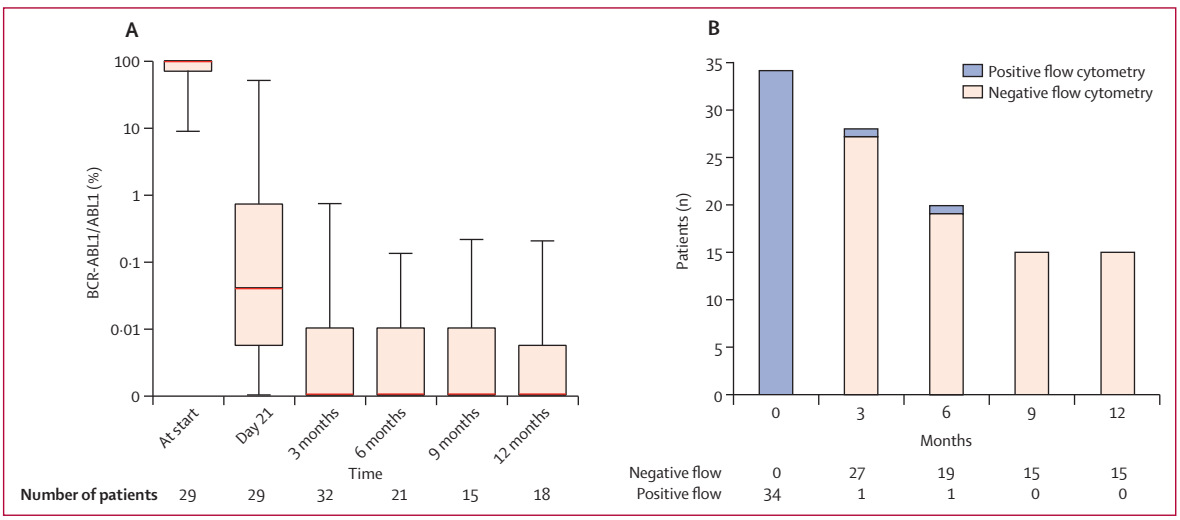

Figure 2: **Minimal residual disease status by PCR and by flow cytometry with follow-up**
(A) Minimal residual disease by time from therapy according to BCR-ABL1/ABL1 percentage. The number of patients at the beginning of the trial is only 29 because three had previous therapy and five did not have a BCR/ABL1 assessment at the beginning, or it was not quantified. The red line is the median at the stated timepoints. Several patients at different time intervals had overlapping values. In one patient, BCR-ABL1 was undetectable at presentation by reverse-transcription quantitative PCR and was detected by FISH. (B) Minimal residual disease by time from therapy according to multiparameter flow cytometry.

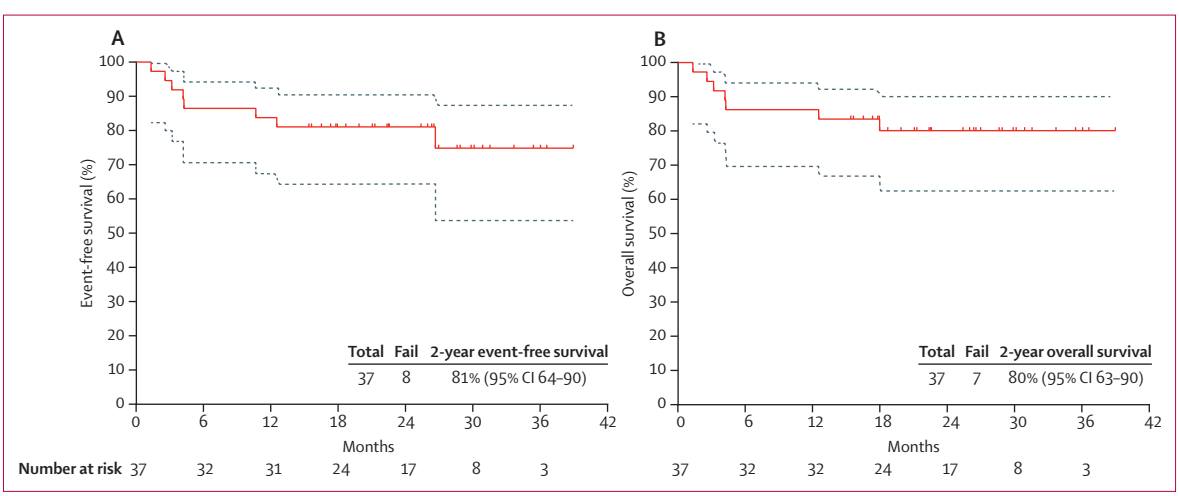

Figure 3: **Event-free survival (A) and overall survival (B) of the patients**
Dotted lines are 95% CIs.

sepsis; two of these deaths were possibly related to study treatment. 13 patients had hypertension; six of the episodes were grade 3 and occurred during either induction or the first consolidation cycles (table 3).

18 (49%) patients had their dose reduced to 30 mg after a median of 13 weeks due to skin rash (n=7), raised aminotransferase concentrations (n=4), deconditioning (n=3), prolonged thrombocytopenia (n=2), pancreatitis (n=1), or pleural or pericardial effusion (n=1). Two patients had further decreases to 15 mg daily after a median of 9 weeks (range 8–11) because of persistently raised aminotransferase concentrations and atrial fibrillation with rapid ventricular response (one patient each). An additional patient was switched to dasatinib after the sixth cycle because of severe bullous skin lesions and another patient was switched to imatinib after the second cycle because of generalised deconditioning and exacerbation of comorbid medical conditions.

After the increased incidence of vascular toxic effects was recognised during the pivotal ponatinib trials in 2013 and based on our own experience with two possible related deaths on-study, we elected to modify our strategy for safety management. We offered our patients the option to switch tyrosine-kinase inhibitors, and for those who elected to stay on ponatinib, we reduced the ponatinib dose to 30 mg and further decreased it to 15 mg in patients in complete molecular response. 13 patients remained on ponatinib, at a dose of 15 mg daily in

12 patients and 30 mg daily in one patient (whose BCR-ABL transcript concentrations were 0·04%). Furthermore, beginning on Aug 1, 2014, the protocol was amended; ponatinib was given at 45 mg daily for 14 days during induction therapy, then at 30 mg daily continuously starting with the second cycle, and then further reduced to 15 mg daily continuously once a complete molecular response was achieved. No further vascular events or any other serious adverse events were recorded in patients receiving lower doses of ponatinib, and none after the amendment of the study protocol. Overall, 11 patients switched from ponatinib to dasatinib (n=8), imatinib (n=2), or nilotinib (n=1). Two patients switched because of side-effects encountered on ponatinib therapy, and nine decided to electively switch tyrosine-kinase inhibitors. At the time of switching, six patients were receiving consolidation and five patients were receiving maintenance therapy. The best response at the time of switch was major molecular response in all 11 patients, with eight (73%) patients in complete molecular response.

Furthermore, we retrospectively compared characteristics and outcome between patients who solely stayed on ponatinib and those who switched tyrosine-kinase inhibitors for the reasons mentioned above. There was no difference in patient characteristics or in outcome (complete remission duration, event-free survival, and overall survival) between patients who remained on ponatinib throughout their treatment and those who were switched to other tyrosine-kinase inhibitors (appendix).

Discussion

In this ongoing phase 2 study, we show that the combination of hyper-CVAD with ponatinib is highly effective, with a major molecular response in 100% (37/37) and complete molecular response in 78% (29/37) of patients. Patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia traditionally have a very poor outcome with chemotherapy, particularly if they did not undergo allogeneic stem cell transplantation in the first complete remission.[26] Combinations of BCR-ABL1 tyrosine-kinase inhibitors with chemotherapy have significantly improved outcomes, especially when the tyrosine-kinase inhibitors are incorporated early and given daily and continuously with chemotherapy.[1–8] Despite the high remission rates obtained with the combination of chemotherapy and first-generation and second-generation tyrosine kinase inhibitors, long-term survival remains at 40–50%, with most relapsing patients acquiring the T315I mutation (30–70%).[2,4,13,14]

After follow-up of over 2 years, only two patients have relapsed, and no T315I mutations have been detected, a significant improvement compared with first-generation and second-generation tyrosine-kinase inhibitors.[27] With a median follow-up of 26 months, 2-year complete remission, event-free survival, and overall survival was 97%, 81%, and 80%, respectively. These results compare favourably with previous experience in similar patients treated with hyper-CVAD and imatinib or dasatinib. The reported 2-year complete remission and overall survival for imatinib were 58% and 67%, respectively, and 70% and 64% for dasatinib.[2,4] Furthermore, our results compare favourably with the recent results of the Group for

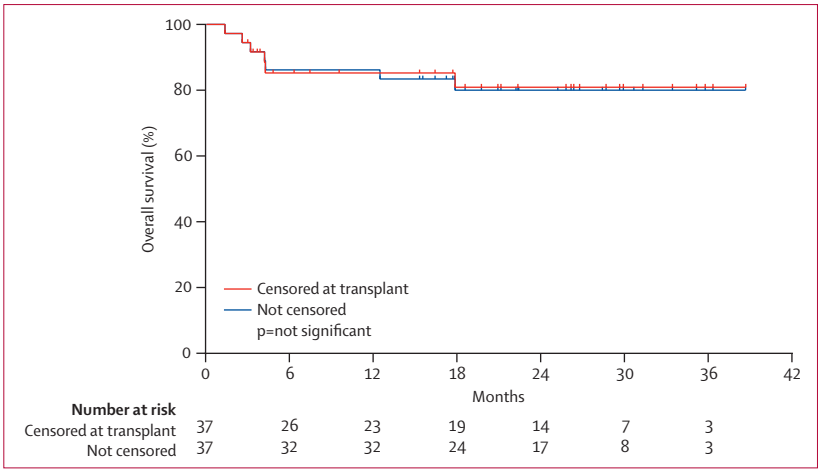

Figure 4: **Overall survival with and without censoring for allogeneic stem-cell transplantation**

	Grade 1–2	Grade 3	Grade 4	Grade 5
Infections overall	0	25 (68%)	6 (16%)	1 (3%)
Infections during induction	0	19 (51%)	1 (3%)	0
Increased alanine aminotransferase or aspartate aminotransferase	22 (59%)	12 (32%)	2 (5%)	0
Increased bilirubin	25 (68%)	7 (19%)	2 (5%)	0
Skin rash	23 (62%)	8 (22%)	0	0
Increased amylase or lipase	5 (14%)	5 (14%)	2 (5%)	0
Hypertension	7 (19%)	6 (16%)	0	0
Pancreatitis	3 (8%)	6 (16%)	0	0
Bleeding	9 (24%)	4 (11%)	0	1 (3%)
Mucositis	12 (32%)	4 (11%)	0	0
Abdominal pain	15 (41%)	3 (8%)	0	0
Nausea	20 (54%)	3 (8%)	0	0
Myocardial infarction*	0	0	0	3 (8%)
Thrombotic events†	4 (11%)	3 (8%)	0	0
Diarrhoea	21 (57%)	2 (5%)	0	0
Vomiting	14 (38%)	2 (5%)	0	0
Renal injury	2 (5%)	2 (5%)	0	0
Anorexia	15 (41%)	1 (3%)	0	0
Pericardial effusion	5 (14%)	1 (3%)	0	0
Constipation	21 (57%)	0	0	0
Dry skin	10 (27%)	0	0	0
Pleural effusion	9 (24%)	0	0	0
Visual changes	9 (24%)	0	0	0

Data are n (%). *All three patients died from myocardial infarction, of which two were unexplained and one was in the context of sepsis. †One renal vein thrombosis and two pulmonary emboli.

Table 3: **Treatment-related toxic effects encountered during induction and consolidation chemotherapy cycles regardless of causality**

See Online for appendix

Research on Adult Acute Lymphoblastic Leukemia (GRAALL) trial[28] in 268 patients treated in a two-group comparison of hyper-CVAD with imatinib and reduced-intensity chemotherapy with imatinib. In that trial, the reported 2-year event-free survival was 55% and 2-year overall survival was 60%. Overall, the regimen was well tolerated. Grade 3 and 4 side-effects were consistent with the known intensity of hyper-CVAD toxicity, including episodes of neutropenic fever, infections, and liver dysfunction. Increased incidence of hypertension and vascular side-effects was reported. Development of vascular occlusive events is a major safety concern of ponatinib, which were reported at a cumulative rate of 27% in the PACE study.[29] These events have been observed with all tyrosine-kinase inhibitors, although with different frequencies.[30] Because ponatinib is a multikinase inhibitor, it is possible that inhibition of certain kinases such as VEGF, FGFR, or PDGFR can promote endothelial dysfunction, which predisposes patients to thromboembolic events.[31,32] However, other mechanisms might play a part. Patients with pre-existing risk factors for atherogenesis or thromboembolic risk are particularly prone to vascular events with ponatinib and warrant close monitoring or alternative tyrosine-kinase inhibitor use.

Pooled data from 683 patients who received ponatinib in phase 1, 2, or first-line trials suggested that dose intensity might be associated with the frequency of adverse events, including cardiovascular events.[33] Therefore, we modified our strategy and recommended that patients either switch tyrosine-kinase inhibitor, or in those who chose to stay on ponatinib, reduce ponatinib dose to 30 mg and further decrease it to 15 mg upon reaching a complete molecular response. In patients who chose to stay on ponatinib at the lower dose, all but one (92%) maintained their response with no additional vascular events observed, confirming the retrospective observation of the relation between dose intensity and vascular events. Similarly, switching to a less potent tyrosine-kinase inhibitor when an optimum response was achieved (complete molecular response) was feasible and safe; only one patient has lost response 6 months after the switch with no resistant mutation detected. No difference in outcome was recorded between patients who remained on ponatinib and those who were switched to other tyrosine-kinase inhibitors. Therefore, an induction strategy with the most potent tyrosine kinase inhibitor and further maintenance at lower doses or with other tyrosine-kinase inhibitors is conceivable. We are clinically testing this strategy in the present trial.

Apart from the increased rates of vascular events encountered, a post-hoc analysis comparing the adverse events encountered with hyper-CVAD and ponatinib to similar trials using hyper-CVAD in combination with other tyrosine kinase inhibitors showed similar rates of other grade 3 and above adverse events. Compared with our previous experience with hyper-CVAD in combination with other tyrosine kinase inhibitors, toxic effects were similar except for higher rates of pancreatitis and vascular events, including hypertension. By contrast, there was less pleural effusion and renal dysfunction and a trend for less infection when compared with the toxic effects encountered with hyper-CVAD and dasatinib (appendix).

Although allogeneic stem-cell transplantation has improved the outcome of patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia,[1,34] this study questions whether responding patients should be referred to allogeneic stem-cell transplantation in first complete remission. Ravandi and colleagues[22] found that the achievement and maintenance of a minimal residual disease-negative status was associated with improved survival in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia treated with combination chemotherapy and tyrosine-kinase inhibitors but without allogeneic stem-cell transplantation. Therefore, monitoring of minimal residual disease might identify patients in first complete remission in whom further consolidation with allogeneic stem-cell transplantation might not be needed.

Our study population is somewhat different than other similar published cohorts. The patient population had a mean white blood cell count that was lower than expected based on similar trials, but the range for this value was very wide in our population. Similarly, the age of our patients is somewhat higher than that seen in other comparable studies. Higher age might be indicative of the nature of the patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia referred to our institution. However, this median is similar to our previous experience with the combination of hyper-CVAD and other tyrosine-kinase inhibitors, in which the median of patients treated was 53 years (range 21–79).[2]

We have shown the feasibility of combining chemotherapy with ponatinib in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia. The regimen is effective in achieving early sustained responses, with a median follow-up of 26 months. In responding patients, long-term disease-free survival was not affected by allogeneic stem-cell transplantation. A longer follow-up is needed to confirm these findings. New strategies, including dose titration of ponatinib, optimum control of vascular risk factors, and the addition of new monoclonal antibodies, might help to further improve outcomes.

Contributors

EJ, HK, and SO'B designed the study, accrued patients, collected data, analysed data, and wrote the manuscript. VJ collected and analysed data. XH did the statistical design of the study protocol. RG and ZB analysed data and wrote the manuscript. FR, DT, SF, NP, ND, GG-M, KS, JC, CCY, JDK, JJ, ZE, MK, TK, NJ, CD, and SO'B accrued patients and collected data.

Declaration of interests

TK reports consulting fees from ARIAD outside the submitted work. JC reports grants and consulting fees from ARIAD during the conduct of the study; grants and consulting fees from Bristol-Myers Squibb, Novartis, and Pfizer outside the submitted work. NP reports consulting fees and honoraria from Incyte, Novartis, and LFB Biotech, and research funding from Novartis and Stemline. WW reports adviser and consulting

fess from Sanofi, Genentech/Roche, Pharmacyclics, Celgene, Gilead, GSK and Novartis, Genzyme, Merck, Abbvie, and Emergent, and contracted research with GSK and Novartis, Abbvie, Genentech, Karyopharm, Pharmacyclics, Ascerta, Gilead, Jansen, Emergent, Juno Therapeutics, and KITE Pharma. The other authors declare no competing interests.

References

1 Fielding AK, Rowe JM, Buck G, et al. UKALLXII/ECOG2993: addition of imatinib to a standard treatment regimen enhances long-term outcomes in Philadelphia positive acute lymphoblastic leukemia. *Blood* 2014; **123:** 843–50.

2 Ravandi F, O'Brien S, Thomas D, et al. First report of phase II study of dasatinib with hyper-CVAD for the frontline treatment of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia. *Blood* 2010; **116:** 2070–77.

3 Schultz KR, Bowman WP, Aledo A, et al. Improved early event-free survival with imatinib in Philadelphia chromosome-positive acute lymphoblastic leukemia: a children's oncology group study. *J Clin Oncol* 2009; **27:** 5175–81.

4 Thomas DA, O'Brien SM, Faderl S, et al. Long-term outcome after hyper-CVA and imatinib (IM) for de novo or minimally treated Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-ALL). *Proc Am Soc Clin Oncol* 2010; **28:** 15 (suppl): abstr 6506.

5 Tanguy-Schmidt A, Rousselot P, Chalandon Y, et al. Long-term follow up of the imatinib GRAAPH-2003 study in newly diagnosed patients with de novo Philadelphia chromosome-positive acute lymphoblastic leukemia: a GRAALL study. *Biol Blood Marrow Transplant* 2013; **19:** 150–55.

6 Bassan R, Rossi G, Pogliani EM, et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. *J Clin Oncol* 2010; **28:** 3644–52.

7 Pfeifer H, Goekbuget N, Volp C, et al. Long-term outcome of 335 patients receiving different schedules of imatinib and chemotherapy as front-line treatment for Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood* 2010; **116:** abstract 173.

8 Wassmann B, Pfeifer H, Goekbuget N, et al. Alternating versus concurrent schedules of imatinib and chemotherapy as front-line therapy for Philadelphia-positive acute lymphoblastic leukemia (Ph+ ALL). *Blood* 2006; **108:** 1469–77.

9 Dombret H, Gabert J, Boiron JM, et al. Outcome of treatment in adults with Philadelphia chromosome-positive acute lymphoblastic leukemia—results of the prospective multicenter LALA-94 trial. *Blood* 2002; **100:** 2357–66.

10 Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science* 2001; **293:** 876–80.

11 Shah NP, Nicoll JM, Nagar B, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell* 2002; **2:** 117–25.

12 Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL. Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* 2004; **305:** 399–401.

13 Rousselot P, Coude MM, Huguet F, et al. Dasatinib and low intensity chemotherapy for first-line treatment in patients with de novo Philadelphia positive ALL aged 55 and over: final results of the EWALL-Ph-01 study. *Blood* 2012; **120** (suppl 1)**:** abstr 666.

14 Foa R, Vitale A, Vignetti M, et al. Dasatinib as first-line treatment for adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia. *Blood* 2011; **118:** 6521–28.

15 O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell* 2009; **16:** 401–12.

16 Cortes JE, Kantarjian H, Shah NP, et al. Ponatinib in refractory Philadelphia chromosome-positive leukemias. *N Engl J Med* 2012; **367:** 2075–88.

17 Cortes JE, Kim DW, Pinilla-Ibarz J, et al. A phase 2 trial of ponatinib in Philadelphia chromosome-positive leukemias. *N Engl J Med* 2013; **369:** 1783–96.

18 Criteria Committee of the New York Heart Association. Nomenclature and criteria for diagnosis of diseases of the heart and great vessels. 9th edn. Little, Brown & Co: Boston, MA, 1994: 253–56.

19 Thomas DA, Faderl S, Cortes J, et al. Treatment of Philadelphia chromosome-positive acute lymphocytic leukemia with hyper-CVAD and imatinib mesylate. *Blood* 2004; **103:** 4396–407.

20 Kantarjian H, Thomas D, O'Brien S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer* 2004; **101:** 2788–801.

21 Thomas DA, O'Brien S, Faderl S, et al. Chemoimmunotherapy with a modified hyper-CVAD and rituximab regimen improves outcome in de novo Philadelphia chromosome-negative precursor B-lineage acute lymphoblastic leukemia. *J Clin Oncol* 2010; **28:** 3880–89.

22 Ravandi F, Jorgensen JL, Thomas DA, et al. Detection of MRD may predict the outcome of patients with Philadelphia chromosome-positive ALL treated with tyrosine kinase inhibitors plus chemotherapy. *Blood* 2013; **122:** 1214–21.

23 Luthra R, Sanchez-Vega B, Medeiros LJ. TaqMan RT-PCR assay coupled with capillary electrophoresis for quantification and identification of bcr-abl transcript type. *Mod Pathol* 2004; **17:** 96–103.

24 Branford S, Fletcher L, Cross NS, et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. *Blood* 2008; **112:** 3330–08.

25 Jones D, Thomas D, Yin CC, et al. Kinase domain point mutations in Philadelphia chromosome-positive acute lymphoblastic leukemia emerge after therapy with BCR-ABL kinase inhibitors. *Cancer* 2008; **113:** 985–94.

26 Weir EG, Cowan K, LeBeau P, Borowitz MJ. A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection. *Leukemia* 1999; **13:** 558–67.

27 Soverini S, De Benedittis C, Papayannidis C, et al. Drug resistance and BCR-ABL kinase domain mutations in Philadelphia chromosome-positive acute lymphoblastic leukemia from the imatinib to the second-generation tyrosine kinase inhibitor era: the main changes are in the type of mutations, but not in the frequency of mutation involvement. *Cancer* 2014; **120:** 1002–09.

28 Chalandon Y, Thomas X, Hayette S, et al. Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia. *Blood* 2015; **125:** 3711–19.

29 Cortes JE, Ibarz JP, Coutre PL, et al. Long-term follow-up of ponatinib efficacy and safety in the phase 2 PACE trial. *Blood* 2014; **124** (suppl)**:** abstract 3135.

30 Valent P, Hadzijusufovic E, Schernthaner G, Wolf D, Rea D, le Coutre P. Vascular safety issues in CML patients treated with BCR/ABL1 kinase inhibitors. *Blood* 2015; **125:** 901–06.

31 Rivera VM PJ, Gonzalvez F, Baker F, Gozgit JM, Hodgson G. Comparative TKI profiling analyses to explore potential mechanisms of ponatinib-associated arterial thrombotic events. *Blood* 2014; **124** (suppl)**:** abstract 1783.

32 Hadzijusufovic E, Albrecht-Schgoer K, Huber K, et al. Nilotinib exerts direct pro-atherogenic and anti-angiogenic effects on vascular endothelial cells: a potential explanation for drug-induced vasculopathy in CML. *Blood* 2013; **122** (suppl)**:** abstract 257.

33 Knickerbocker R, Haluska FG, Baccarani M, Cortes J, Hochhaus A, Talpaz M. Impact of dose intensity of ponatinib on selected adverse events: multivariate analyses from a pooled population of clinical trial patients. *Blood* 2014; **124** (suppl)**:** abstract 4546.

34 Fielding AK. Philadelphia-positive acute lymphoblastic leukemia–is bone marrow transplant still necessary? *Biol Blood Marrow Transplant* 2011; **17:** S84–88.