Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study

Jan A Burger, Michael J Keating, William G Wierda, Elena Hartmann, Julia Hoellenriegel, Nathalie Y Rosin, Iris de Weerdt, Ghayathri Jeyakumar, Alessandra Ferrajoli, Marylou Cardenas-Turanzas, Susan Lerner, Jeffrey L Jorgensen, Graciela M Nogueras-González, Gracy Zacharian, Xuelin Huang, Hagop Kantarjian, Naveen Garg, Andreas Rosenwald, Susan O'Brien

Summary

Background **Ibrutinib, an orally administered covalent inhibitor of Bruton's tyrosine kinase (BTK), is an effective treatment for relapsed chronic lymphocytic leukaemia (CLL). We investigated the activity and safety of the combination of ibrutinib with the monoclonal antibody rituximab in patients with high-risk CLL.**

Methods **In this single-arm phase 2 study, we enrolled adult patients with high-risk CLL at the MD Anderson Cancer Center (Houston, TX, USA). All enrolled participants had high-risk cytogenetic abnormalities (deletion 17p, *TP53* mutation, or deletion 11q) or a short progression-free survival (PFS <36 months) after previous first-line chemoimmunotherapy. Patients with symptomatic disease requiring therapy received 28-day cycles of once-daily ibrutinib 420 mg together with rituximab (375 mg/m², intravenously, every week during cycle 1, then once per cycle until cycle 6), followed by continuous daily single-agent ibrutinib 420 mg until disease progression or until toxicities or complications precluded further treatment. The primary endpoint was progression-free survival in the intention-to-treat population. This study is registered with ClinicalTrials.gov number NCT01520519, and is no longer accruing patients.**

Findings **Between Feb 28, 2012, and Sept 11, 2012, we enrolled 40 patients with CLL with high-risk disease features, 20 of whom had deletion 17p (del[17p]) or *TP53* mutations (16 previously treated, four untreated), 13 had relapsed CLL with deletion 11q (del[11q]), and seven a PFS less than 36 months after first-line chemoimmunotherapy. 18-month PFS in all patients was 78·0% (95% CI 60·6–88·5), whereas in those with a del(17p) or *TP53* mutation it was 72·4% (45·6–87·6) Toxicity was mainly mild to moderate in severity (grade 1–2). Diarrhoea occurred in ten (25%) patients (grade 1 in nine patients and grade 2 in one), bleeding events in 14 (33%) patients (eight grade 1 and five grade 2), nausea or vomiting in 15 patients (38%) (ten grade 1 and five grade 2), and fatigue in seven (18%) patients (four grade 1 and three grade 2). Five patients (13%) had grade 3 infections (two lung infections, one upper respiratory tract infection, one sepsis, and one mucositis), and no grade 4 or 5 infections occurred. One patient had grade 4 neutropenia.**

Interpretation **The encouraging safety and activity of ibrutinib and rituximab in this population of patients with high-risk CLL merits further investigation of this combination.**

Funding **Pharmacyclics Inc, Cancer Prevention and Research Institute of Texas, Leukemia and Lymphoma Society, National Cancer Institute, MD Anderson Cancer Center.**

Introduction

Treatment of patients with chronic lymphocytic leukaemia (CLL) is undergoing major changes[1] because of the emergence of new treatment approaches, such as kinase inhibitors targeting B-cell receptor signalling[2,3] and novel monoclonal antibodies.[4] Patients with CLL with high-risk cytogenetic abnormalities (deletion 17p, *TP53* mutation, or deletion 11q)[5] can especially benefit from new kinase inhibitors, in view of the high proportion of responses noted in patients treated with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib[2] and the PI3Kδ inhibitor idelalisib in combination with rituximab.[3] Chemoimmunotherapy regimens, such as fludarabine, cyclophosphamide, and rituximab,[6] or bendamustine in combination with rituximab,[7] are effective options for younger patients with lower risk CLL. However, patients with del(17p) or *TP53* mutations respond poorly to chemoimmunotherapy, typically have short periods of remission,[6,7] and consequently a short median life expectancy of only 2–3 years after first-line therapy.[8] Patients with relapsed CLL and del(11q), who often have extensive lymphadenopathy,[9] also have a poor prognosis. These patients respond quite well to chemoimmunotherapy regimens, but have shorter remissions than do low-risk patients.[9,10] Finally, patients with CLL who relapse early after first-line chemoimmunotherapy (ie, after <24–36 months) are another challenging group of high-risk patients[8,10] with a low proportion of responses and short survival times when retreated with chemoimmunotherapy.[8,10]

Ibrutinib (previously called PCI-32765) is a potent (IC_{50} 0·5 nmol/L) selective BTK inhibitor that inactivates BTK through irreversible covalent bonding to Cys-481 in the ATP-binding domain of the kinase.[11] Ibrutinib is given to

Lancet Oncol 2014

Published Online
August 21, 2014
http://dx.doi.org/10.1016/S1470-2045(14)70335-3

See Online/Comment
http://dx.doi.org/10.1016/S1470-2045(14)70388-2

Department of Leukemia (J A Burger MD, Prof M J Keating MD, Prof W G Wierda MD, J Hoellenriegel MS, N Y Rosin PhD, I de Weerdt MD, G Jeyakumar MD, A Ferrajoli MD, M Cardenas-Turanzas PhD, S Lerner MS, G Zacharian MS, Prof H Kantarjian MD, Prof S O'Brien MD), **Department of Hematopathology** (J L Jorgensen MD), **Department of Biostatistics** (G M Nogueras-González MS, X Huang PhD), **and Department of Diagnostic Radiology** (N Garg MD), **MD Anderson Cancer Center, Houston, TX, USA; and Institute of Pathology, University of Würzburg, Würzburg, Germany** (E Hartmann MD, Prof A Rosenwald MD)

Correspondence to:
Dr Jan A Burger, Department of Leukemia, Unit 428, The University of Texas MD Anderson Cancer Center, PO Box 301402, Houston, TX 77230-1402, USA
jaburger@mdanderson.org

patients with CLL once daily orally at a fixed dose of 420 mg continuously until disease progression or toxicity. Early-stage clinical trials showed single-agent ibrutinib to be especially active in patients with CLL[2,12] and mantle cell lymphoma.[13] The CLL data are based on a phase 1b–2 multicentre study of ibrutinib in 85 patients with relapsed or refractory CLL or small lymphocytic lymphoma.[2] The authors reported a complete or partial response in 60 (71%) of 85 patients, and an additional five (15%) of 34 patients in the 840 mg cohort and ten (20%) of 51 in the 420 mg cohort had a partial response with lymphocytosis. The responses were independent of clinical and genomic risk factors present before treatment, including advanced-stage disease, number of previous treatments, or presence of 17p deletion. At 26 months, the estimated progression-free survival (PFS) was 75% and overall survival was 83%. The results from this study led the US Food and Drug Administration (FDA) to designate ibrutinib as a breakthrough therapy in patients with CLL with del(17p) and their approval of single-agent ibrutinib for previously treated patients with CLL in February, 2014.[14] In another recent randomised trial,[15] investigators compared ibrutinib with the anti-CD20 antibody ofatumumab in patients with relapsed or refractory CLL or small lymphocytic lymphoma. Objective responses were significantly more common in the ibrutinib group (43%) than in the ofatumumab group (4%). A further 20% of ibrutinib-treated patients had a partial response with lymphocytosis. At a median follow-up of 9·4 months, ibrutinib significantly improved PFS and overall survival when compared with ofatumumab.[15]

In patients with CLL, ibrutinib mobilises leukaemia cells from tissue sites into the peripheral blood,[16] resulting in lymphocytosis during the first few weeks of treatment, which is variable among patients and is directly related to the presence of the drug. On an intermittent dosing schedule, a raised absolute lymphocyte count rapidly dropped during the off-ibrutinib period, presumably because of increased tissue homing, and then increased again when ibrutinib treatment was restarted.[12] This lymphocytosis is asymptomatic and normalises after a median of 6·2 months.[17] It is caused by the re-distribution of CLL cells from the tissue compartments into the peripheral blood[12,16] rather than by disease progression. Preclinical models show that ibrutinib inhibits CLL cell survival and proliferation,[18] and leukaemia cell migration towards tissue-homing chemokines (CXCL12 and CXCL13) and integrin-mediated CLL cell adhesion;[19,20] this process resulted in transient redistribution lymphocytosis into the peripheral blood in an adoptive transfer CLL mouse model.[19] In view of this redistribution with single-agent ibrutinib, which can persist in many patients, the well-recognised efficacy of rituximab in clearing leukaemia cells from the peripheral blood, and the documented benefit of rituximab in chemotherapy combinations on PFS and overall survival in untreated patients with CLL,[6] we explored the safety and activity of the combination of ibrutinib and rituximab in patients with high-risk CLL.

Methods

Study design and participants

Adult patients with high-risk CLL or small lymphocytic lymphoma were eligible for enrolment in this single-arm, phase 2 study. Patients had to be aged 18 years or older and have previously treated high-risk CLL or untreated CLL with del(17p) or *TP53* mutation. High-risk CLL was defined by the presence of a 17p deletion, *TP53* mutation, or 11q deletion, according to the hierarchical cytogenetic model developed by Dohner and colleagues.[5] Patients with CLL and small lymphocytic lymphoma who had short remission durations of less than 36 months after previous first-line chemoimmunotherapy also fulfilled criteria for high-risk disease. Patients with a 17p deletion or *TP53* mutation were not required to have received previous treatment because of the poor outcome of such patients with chemoimmunotherapy. For previously treated patients, a 30-day washout period since previous therapy was required. Patients had to have an indication for therapy in accordance with the 2008 International Workshop on Chronic Lymphocytic Leukemia (IWCLL) criteria,[21] adequate renal and hepatic function (defined by an estimated creatinine clearance of >30 mL/min), an alanine aminotransferase concentration of no more than 2·5 times the upper limit of the normal range, and no active infection. Patients with uncontrolled autoimmune haemolytic anaemia or autoimmune thrombocytopenia, severe hematopoietic insufficiency (absolute neutrophil count $<0{\cdot}5\times10^9$ cells per L and/or platelet count $<30\times10^9$ cells per L), bleeding diathesis or coagulopathy, recent haemorrhagic events or surgery, and those receiving concomitant treatment with warfarin, were excluded.

This trial was approved by the University of Texas MD Anderson Cancer Center institutional review board. Written informed consent was obtained from all patients in accordance with institutional guidelines and the Declaration of Helsinki.

Procedures

Pretreatment assessment of participants included complete medical history; physical examination; complete blood count with differential white blood cell count; and chemistry profile assessment of serum creatinine, electrolytes, albumin, calcium, uric acid, lactate dehydrogenase, and alanine aminotransferase. We also measured concentrations of serum immunoglobulins and β_2-microglobulin, and analysed the peripheral T-cell lymphocyte subset by flow cytometry. We did bone marrow aspiration before treatment, including infiltration assessment, immunophenotype by flow cytometry, and immunoglobulin heavy chain gene mutation analysis by PCR. Standard metaphase karyotype analysis and genomic abnormalities were detected by fluorescence

in-situ hybridisation with standard CLL probes (Abbott Molecular, Des Plaines, IL, USA).

Treatment consisted of oral ibrutinib 420 mg continuously daily (one cycle was 28 days), in combination with weekly rituximab (375 mg/m² intravenously) for weeks 1–4 (cycle 1) to front-load during the time of highest intravascular CLL cell burden, and then rituximab was given once every 4 weeks until cycle 6, followed by single-agent ibrutinib 420 mg continuously daily. Patients remained on treatment until disease progression or until toxicities or complications precluded further treatment, whichever occurred first. In the event of any grade 3–4 toxicity, ibrutinib was stopped until the adverse event returned to baseline or resolved completely. In the event of any grade 4 toxicity or for any rituximab-related, clinically significant, unmanageable grade 3 adverse events, rituximab was withheld until the adverse event returned to baseline or resolved completely. Dose modifications were not allowed. Clinical and laboratory assessments were done every week during cycle 1, then once every 4 weeks until cycle 6, and then every 3 months thereafter while patients remained on study. Clinical assessments included radiological examinations with CT scans of the chest, abdomen, and pelvis at baseline. Bone marrow aspirations and biopsies were undertaken and assessed by morphology and flow cytometry at baseline and after 3, 6, and 12 months, and once every 12 months thereafter. Minimal residual disease was assessed with four-colour flow cytometry. Quantitative minimal residual disease results were categorised as positive (≥0·01%) or negative (<0·01%).[22]

Safety monitoring was done weekly for the first month, then monthly until month 6, and every 3 months thereafter. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Haematological toxic effects were graded according to the IWCLL system.[21] The European Organisation for the Research and Treatment of Cancer (EORTC) QLQ-C30 questionnaire was used to assess health-related quality of life, as detailed in appendix p 1.

We tested BTK target occupancy during ibrutinib and rituximab treatment using a fluorescently tagged derivative of ibrutinib (PCI-33380). Chemotaxis of CLL cells was assayed across polycarbonate Transwell inserts (Corning Incorporated Life Sciences, Tewksbury, MA, USA). We assessed plasma concentrations of CCL3 and CCL4 before and during treatment by ELISA using Quantikine kits (R&D Systems, Minneapolis, MN, USA). For all other cytokines, we used multiplex bead assays according to the manufacturer's instructions (EMD Millipore, Billerica, MA, USA). For gene-expression analyses, we isolated RNA from CD19-purified CLL cells from the peripheral blood of ten randomly selected patients, collected at baseline and after 7 and 28 days on ibrutinib and rituximab combination treatment. We tested gene expression with HG U133 plus 2.0 oligonucleotide arrays from Affymetrix (Santa Clara, CA, USA) in eight patients samples (two patients had insufficient amounts of RNA), following Affymetrix standard protocols. Further details are provided in appendix pp 1–2.

	All participants (n=40)
Age (years)	63·2 (35–82)
Sex	
Women	14 (35%)
Men	26 (65%)
Rai stage	
I	7 (18%)
II	4 (10%)
III	6 (15%)
IV	23 (58%)
Cytogenetics	
Del(17p) or *TP53* mutation, previously treated	16 (40%)
Del(17p) or *TP53* mutation, previously untreated	4 (10%)
Del(11q)	13 (33%)
Del(13q)	5 (13%)
FISH negative	2 (5%)
IGHV gene status	
Unmutated	32 (80%)
Mutated	1 (3%)
Unknown	7 (18%)
Early relapse after chemoimmunotherapy	7 (18%)
Number of previous treatments	1·9 (1–3)
Time to relapse (months)	14·4 (2–24)
Previous treatments	2·0 (0–8)
Fludarabine, cyclophosphamide, and rituximab	28 (70%)
Monoclonal antibodies (rituximab, ofatumumab, alemtuzumab)	18 (45%)
Bendamustine and rituximab	9 (23%)
Other treatment regimen	25 (63%)
Number of treatment cycles completed	15 (2–21)
Haemoglobin concentration (g/dL)	11·7 (6·7–15·6)
Platelet count (×10⁹ per L)	91·5 (36–242)
White blood cell count (×10⁹ per L)	22·4 (2·2–297·8)
Absolute lymphocyte count (×10⁹ per L)	19·9 (0·4–277)
β_2-microglobulin concentration (mg/L)	4·15 (2·2–12·3)

Data are median (range) or n (%).

Table 1: **Baseline characteristics**

See Online for appendix

Outcomes

The primary endpoint was PFS, which was defined as the time between initiation of study treatment and the date of first documented disease progression or death from any cause. Secondary endpoints were overall survival, the proportion of patients with an overall response, and responses and survival in cytogenetic subgroups, rates of treatment-related and unrelated adverse events, quality of life, and changes in correlative biomarkers during treatment. We assessed responses according to the IWCLL criteria,[21] with the exception that lymphocytosis was not the sole criterion for disease progression. Patients with

persistent lymphocytosis, who were otherwise categorised as a partial response by all other measures, were deemed to have a partial response with lymphocytosis.

To assess for disease progression on treatment, the best response—ie, the true nadir—was considered as baseline. For example, if a patient had normalisation or major reduction of absolute lymphocyte count on ibrutinib and rituximab therapy, but later developed progressive lymphocytosis, this patient would be judged to have progressive disease, especially in the context of other signs (clinical and laboratory) of disease progression, such as progressive cytopenias, increasing amounts of lactate dehydrogenase, organomegaly, progressive lymphadenopathy, or all of these signs.

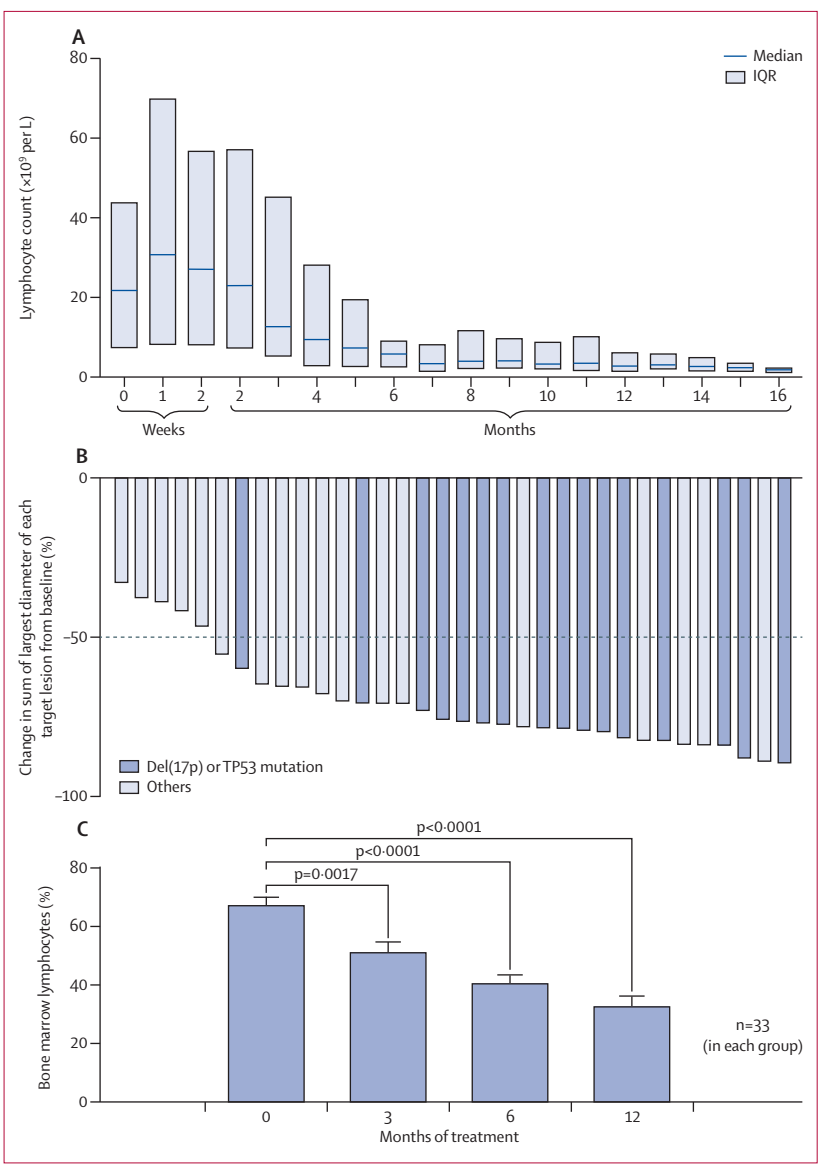

Figure 1: **Change in absolute lymphocyte counts (A), lymph node sizes (B), and bone marrow lymphocytes (C) during treatment**

(A) Time course of absolute lymphocyte counts (median and IQR) in patients with CLL during treatment with ibrutinib and rituximab. (B) Best responses in 34 evaluable patients assessed by CT scan for change from baseline in the sum of the largest diameter of each target lesion. The dashed line shows the percentage change that represents the criterion for lymphadenopathy response. (C) Mean (SE) relative numbers of bone marrow lymphocytes before therapy and after 3, 6, and 12 months of ibrutinib and rituximab combination treatment.

Statistical analysis

We established that a sample size of 40 patients would give us power of at least 80% to determine that the combination of rituximab and ibrutinib improves the PFS in high-risk CLL by 2 months when compared with available data for a similar group of patients treated with standard chemoimmunotherapy.[10] PFS was monitored continuously, and accrual was to have been terminated early if the probability of improving PFS by 2 months was less than 0·01. The Department of Biostatistics at the MD Anderson Cancer Center provided and maintained a website for enrolment of patients into this study and for the continuous evaluation of PFS.

We used descriptive statistics to summarise the patient cohorts at predefined timepoints. We used Fisher's exact test and Wilcoxon rank test in the univariate analyses to compare groups. We used the Wilcoxon signed-rank test for paired data to establish whether there were differences in the distribution between timepoints in the quality-of-life outcomes. The safety analysis was done based on the intent-to-treat population, whereas the efficacy analysis was done using only the evaluable cohort (ie, patients with sufficient follow-up of at least 3 months). Toxicity was reported by type, frequency, and severity. We tabulated the worst toxicity grades per patient for selected adverse events and laboratory measurements. We estimated survival with the Kaplan–Meier method. Patients were censored for PFS at the last clinical assessment before receipt of new anti-leukaemia treatment, or after loss to follow-up, whichever occurred first. Patients were censored for overall survival at the last known alive date.

STATA/SE version 12.1 and GraphPad Prism version 6.00 for Windows were used for statistical analyses. This study is registered with ClinicalTrials.gov, number NCT01520519.

Role of the funding source

The study was supported by Pharmacyclics Inc. Together with Pharmacyclics, the clinical investigators were responsible for the design of the study protocol and analysis plan. The investigators and their research teams gathered, compiled, and analysed all the data. The investigators had full access to the data and analyses for compilation of this report. JAB wrote the first draft, which was carefully reviewed by Pharmacyclics Inc and all coauthors, who approved the final submitted version. The corresponding author had full access to all the data and had final responsibility to submit for publication.

Results

Between Feb 28, 2012, and Sept 11, 2012, 40 patients were enrolled at the MD Anderson Cancer Center, of whom 36 had previously treated high-risk CLL and four had

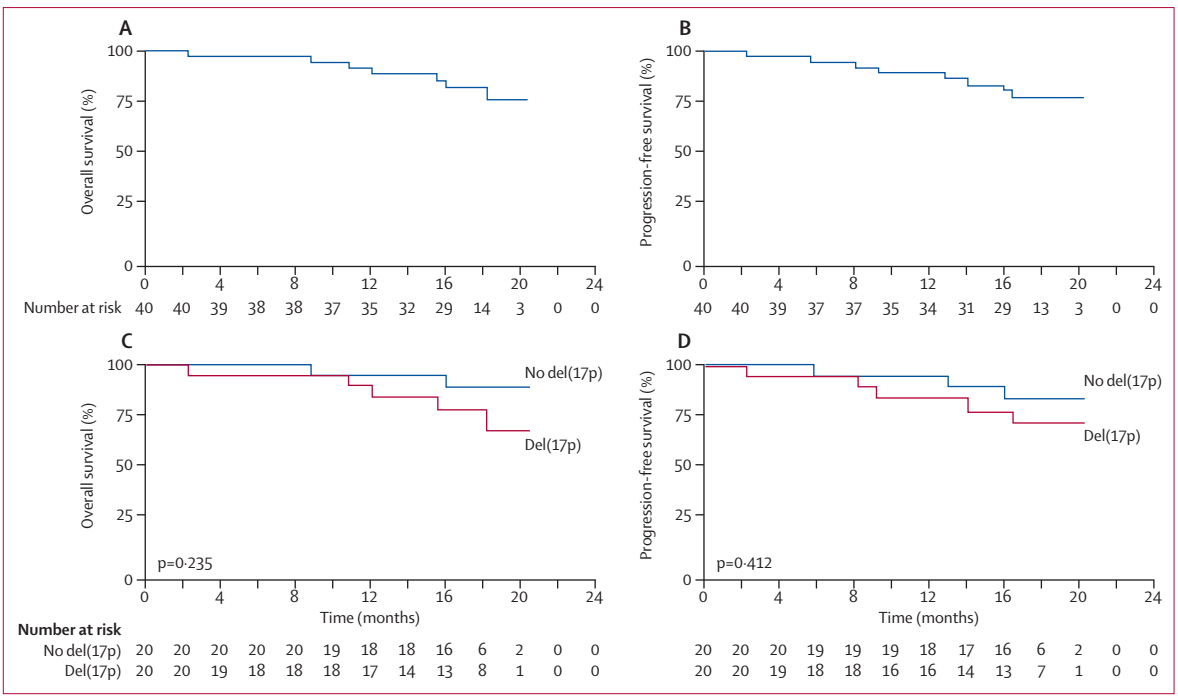

Figure 2: **Kaplan–Meier curves for overall survival and progression-free survival**
(A) Overall survival and (B) progression-free survival for all 40 patients. (C) Overall survival and (D) progression-free survival by del(17p) or *TP53* mutation status.

del(17p) or *TP53* mutation and were previously untreated. This analysis shows the final data from our study, which we extracted on Nov 18, 2013.

Table 1 summarises the baseline characteristics. At a median follow-up of 16·8 months (IQR 2·3–20·5), 31 patients continued on therapy (including 14 of the 20 with del[17p] or *TP53* mutation) and nine (22·5%) discontinued treatment. Of the nine patients who discontinued, two died during the study and six died after study discontinuation. One patient who died during the study had an unrelated infectious complication (pneumonia and CNS abscesses) during the second cycle of treatment, and the other patient died when in remission from an unknown cause. The six other patients who died after study discontinuation died from infectious complications or disease progression (see appendix p 4 for details). Two patients (5%) discontinued treatment because of toxicity (one grade 3 mucositis and one grade 3 recurrent ear and pulmonary infections) after five cycles and after two cycles, respectively. One patient stopped treatment because of resistant pneumonia and another because of progressive chronic obstructive pulmonary disease. Three (8%) patients had disease progression after 176, 395, and 429 days, one of whom had Richter's transformation. The post-remission treatments are detailed in appendix p 4.

39 patients were evaluable for response assessment according to 2008 IWCLL guidelines, whereas one was not evaluable because the follow-up was too short (<3 months). 34 (87%) achieved partial remission, and three (8%) complete remission as their best response; thus 37 (95%) of patients had an overall response. Two patients did not respond to treatment: these were the two patients who discontinued treatment early. Two of the three patients

	Mean (SD) score at baseline (n=39)	Mean (SD) score at 6 months (n=36)	Mean difference (95% CI)	p value
Global health status	70·9 (18·8)	84·2 (12·4)	13·3 (6·1 to 20·5)	<0·0001
Functioning scale				
Physical	88·0 (17·8)	97·6 (6·8)	9·6 (3·6 to 15·6)	0·003
Role	90·2 (19·0)	98·1 (7·7)	7·9 (1·4 to 14·4)	0·02
Social	81·6 (22·9)	97·2 (8·4)	15·6 (7·9 to 23·3)	<0·0001
Emotional	83·8 (14·5)	97·7 (5·8)	13·9 (9·0 to 18·2)	<0·0001
Cognitive	92·3 (12·6)	98·6 (4·7)	6·3 (2·1 to 10·5)	0·006
Symptom scale				
Nausea/vomiting	20·5 (14·0)	1·4 (4·7)	−19·1 (−23·8 to −14·4)	<0·0001
Pain	13·2 (25·1)	3·7 (12·7)	−9·5 (−18·4 to −0·6)	0·04
Fatigue	24·8 (23·7)	4·9 (13·4)	−19·9 (−28·5 to −11·3)	<0·0001
Single-item scale				
Insomnia	24·8 (26·2)	7·4 (16·1)	−17·4 (−29·2 to −7·6)	<0·0001
Appetite loss	10·2 (17·4)	0	−10·2 (−15·7 to −4·7)	<0·0001
Diarrhoea	2·6 (9·0)	11·1 (19·5)	8·5 (1·53 to 15·5)	<0·0001
Constipation	6·0 (15·0)	1·8 (11·1)	−4·2 (−10·1 to 1·74)	0·18
Dyspnoea	19·6 (27·3)	2·8 (9·3)	−16·8 (−25·9 to −7·7)	<0·0001
Financial consequences	12·0 (20·9)	3·7 (10·6)	−8·3 (−15·7 to −0·9)	0·04

Data derived from various measures on the EORTC quality of life questionnaire C30 version 3. p values correspond to comparison of *Z* scores. Significant improvements occurred in global health status, functioning, and symptom scales, and other single items (insomnia, appetite loss, and dyspnoea).

Table 2: **Quality of life at baseline and after 6 months of treatment with ibrutinib and rituximab**

	Grade 1	Grade 2	Grade 3	Grade 4
Lung infection	5 (13%)	9 (23%)	2 (5%)	..
Upper respiratory tract infection	3 (8%)	10 (25%)	1 (3%)	..
Diarrhoea	9 (23%)	1 (3%)	..	..
Neutropenia	..	..	1 (3%)	1 (3%)
Fatigue	4 (10%)	3 (8%)	..	..
Nausea/vomiting/acid reflux	10 (25%)	5 (13%)	..	..
Arthralgia	8 (20%)	3 (8%)	..	..
Aminotransferase increase	..	..	1 (3%)	..
Bleeding events (bruising/rash/epistaxis)	8 (20%)	5 (13%)	..	..
Peripheral neuropathy	1 (3%)	1 (3%)	1 (3%)	..
Weight gain	..	4 (10%)	..	..
Eye disorders (itching/watery eyes)	1 (3%)	2 (5%)	..	..
Mucositis	1 (3%)	1 (3%)	1 (3%)	..
Constipation	1 (3%)	..	..	..
Alopecia	1 (3%)	..	..	..
Atrial fibrillation	1 (3%)	1 (3%)	..	..
Urinary tract infection	..	3 (8%)	..	..
Insomnia	..	4 (10%)	..	..
Headache	3 (8%)	..	..	..
Anaemia	1 (3%)	2 (5%)	..	..
Osteoporosis	..	1 (3%)	..	..
Hot flushes	..	2 (5%)	..	..
Constipation	1 (3%)	2 (5%)	..	..
Anxiety	..	2 (5%)	..	..
Dry mouth	1 (3%)	1 (3%)	..	..
Dyspnoea	..	1 (3%)	..	..
Subdural haematoma	..	..	1 (3%)	..
Sepsis	..	..	1 (3%)	..

Table 3: **Adverse events**

who achieved complete remission had del(17p) or *TP53* mutation and were previously untreated. One patient in complete remission was negative for minimal residual disease by flow cytometry. 16 partial remissions and two complete remissions were noted in the 20 patients with del(17p) or *TP53* mutation.

The median time to achieve a response was 5·72 months (IQR 5·32–6·87), and the median duration of response was 15·44 months (10·55–18·27). In the 38 patients evaluable for lymphocyte count, blood lymphocytosis peaked at a median of 1 week of combination therapy, and then decreased continuously (figure 1A). Lymphocyte count normalised (absolute lymphocyte count <4×10^9 cells per L) after 7 months (figure 1A). CT scans at 6 months and 12 months showed a greater than 50% reduction in lymphadenopathy in 29 (85%) of the 34 patients with measurable lesions both at and after baseline, and that all 34 patients had improvements in lymphadenopathy (figure 1B). Notable reductions were also recorded in spleen size (appendix p 18). Serial bone marrow assessments showed a significant decrease in bone marrow infiltration by CLL cells (figure 1C).

18-month PFS for all patients was 78·0% (95% CI 60·6–88·5); in patients with del(17p) or *TP53* mutation, 18-month PFS was 72·4% (45·6–87·6). 18-month overall survival was 83·8% (95% CI 67·2–92·4) for all patients, whereas for patients with del(17p) or *TP53* mutation, it was 78·4% (52·0–91·4, figure 2).

Sustained improvement in cytopenias, defined as improvement by more than 50% from baseline or a haemoglobin concentration higher than 110 g/L, or a platelet count higher than 100×10^9 cells per L lasting for at least two cycles without transfusion or administration of growth factors, was noted in 15 (63%) of 24 patients with baseline thrombocytopenia, and 15 (88%) of 17 with anaemia (appendix p 19). Because of the early resolution of lymphocytosis, cases of partial remissions with lymphocytosis were rare at early timepoints (three [8%] patients after six cycles) and absent after 12 months of treatment.

In a comparison of differences in health-related quality of life between baseline and during treatment, we noted that patients who received ibrutinib and rituximab showed significant improvements in overall health and quality of life after 6 and 12 months (table 2, and appendix p 3), which coincided with a significant weight gain at 3 and 6 months (appendix p 20).

Overall, treatment was well tolerated, with respiratory infections (11 cases of pneumonia [grade 2 or 3] and ten cases of upper respiratory infections [grade 2] and one grade 3 upper respiratory infection) being the most common adverse events. Possibly treatment-related grade 1–3 adverse events of mucositis (in three patients) and peripheral neuropathy (three patients) also occurred. One grade 3 subdural hematoma occurred in a 70-year-old man, who fell and subsequently developed headaches. This patient did not require any intervention; ibrutinib was initially withheld, and later restarted. Milder toxicities included grade 1–2 diarrhoea (n=10); bruising and rash (n=13); nausea, vomiting, or acid reflux (n=15); fatigue (n=7); and bone pain, myalgia, or arthralgia (n=11). Haematological toxicities included grade 3–4 neutropenia (n=2) and grade 2 anaemia (n=2). The toxicities are summarised in table 3 and appendix p 21; appendix p 5 shows toxicities stratified by whether or not they were judged to be treatment related.

We recorded a relatively homogeneous gene-expression response of CLL cells during combination therapy. Appendix p 23 shows heatmaps that illustrate the most downregulated genes in CLL cells after 7 days and 28 days of treatment compared with baseline gene expression. The genes with known and potentially relevant function are annotated on appendix p 23, and include the chemokine CCL3; EGR1, 2, and 3; and CD72. We provide more details in the appendix pp 6–17, where we display the most upregulated and downregulated genes after 7 days and 28 days of therapy.

Mean β_2-microglobulin concentration in the 40 patients before treatment was 5·2 mg/L (SE 0·43), and decreased significantly during ibrutinib and rituximab treatment to 3·2 mg/L (SE 0·22) after 3 months (n=37), to 2·9 mg/L (0·21) after 6 months (n=35), to 2·5 mg/L (0·15) after

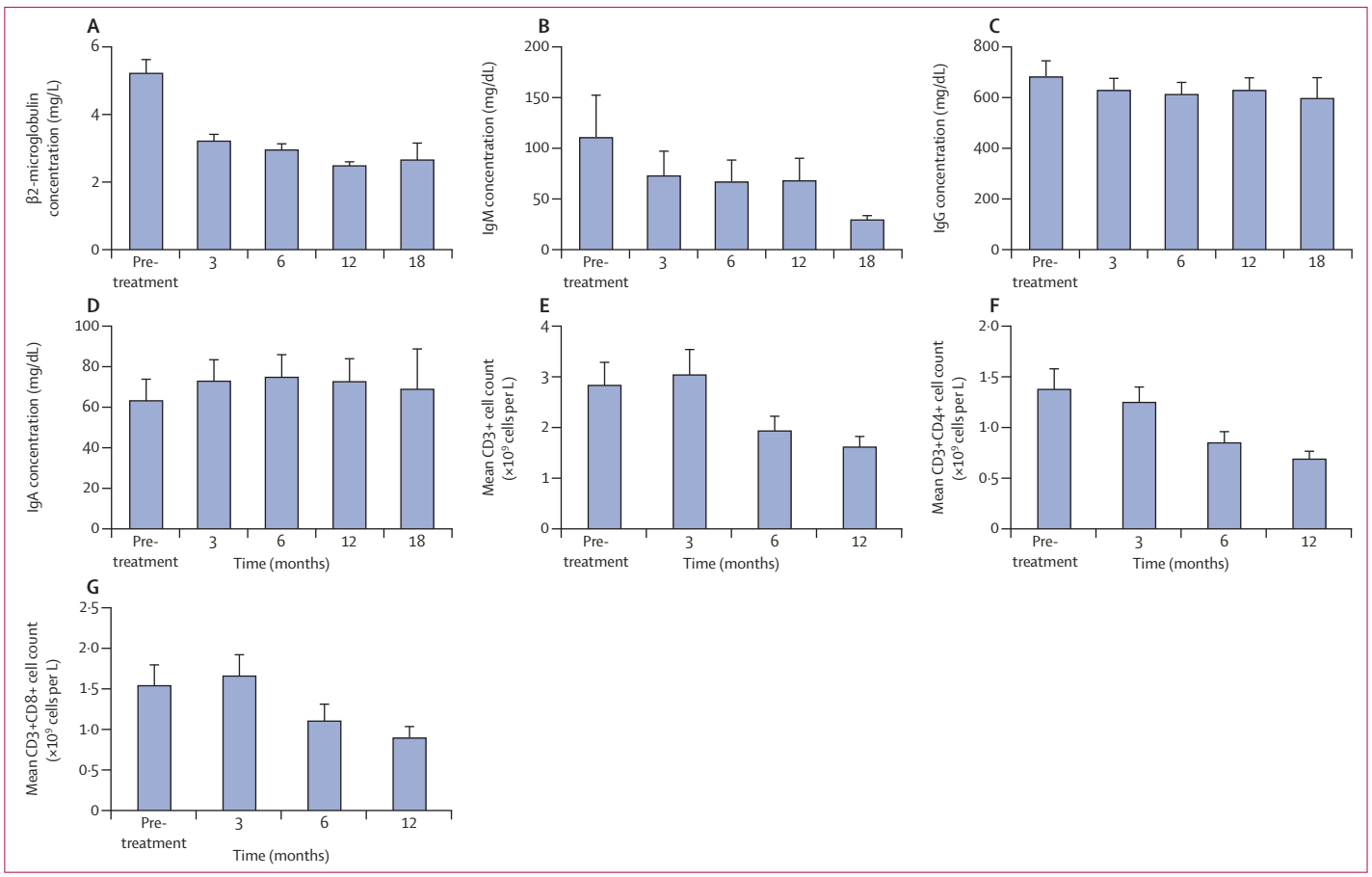

Figure 3: **Changes in β_2-microglobulin concentration (A), immunoglobulin levels (B, C, D), and T-cell subsets (E, F, G) during treatment**

12 months (n=35), and to 2·6 mg/L (0·53) after 18 months (n=5; figure 3A). That the concentrations plateau rather than normalise over time might be because of residual disease. IgG and IgA concentrations did not change significantly during ibrutinib and rituximab therapy during 18 months of follow-up (figure 3C, D). We noted a decrease in IgM levels, which was not statistically significant (figure 3B). Mean pretreatment IgM concentrations of 110 mg/dL (SE 42·7) (n=39) fell to 72 mg/dL (25·3) after 3 months (n=37), to 66 mg/dL (SE 22·8) after 6 months (n=36), to 66 mg/dL (23·2) after 12 months (n=35), and to 29 mg/dL (SE 4·8) after 18 months (n=4). T cells and T-cell subset numbers also changed during ibrutinib and rituximab therapy (figure 3E–G). Increased numbers of CD3+ cells before treatment (mean $2{\cdot}82\times10^9$ cells per L [SE 0·48], n=30) remained at $3{\cdot}029\times10^9$ cells per L (0·52) after 3 months (n=36), but then decreased to $1{\cdot}919\times10^9$ cells per L (SE 0·31) after 6 months (n=33), and to $1{\cdot}606\times10^9$ cells per L (0·22) after 12 months (n=32). This reduction was due to changes in both CD4+ and CD8+ T cells. Mean CD3+CD4+ cell counts decreased significantly during ibrutinib and rituximab therapy from $1{\cdot}369\times10^9$ cells per L (SE 0·21) before treatment (n=35) to $1{\cdot}240\times10^9$ cells per L (0·16) after 3 months (n=37), to $0{\cdot}841\times10^9$ cells per L (0·12) after 6 months (n=33), and to $0{\cdot}681\times10^9$ cells per L (0·08) after 12 months (n=32, p=0·014). Mean CD3+CD8+ cell counts increased slightly from $1{\cdot}527\times10^9$ cells per L (SE 0·27) before therapy (n=35) to $1{\cdot}649\times10^9$ cells per L (0·27) after 3 months (n=37), but then declined to $1{\cdot}090\times10^9$ cells per L (SE 0·22) after 6 months (n=33) and $0{\cdot}885\times10^9$ cells per L (0·15) after 12 months (n=32). Appendix p 22 provides T-cell counts in individual patients before and during ibrutinib and rituximab therapy.

The combination of ibrutinib and rituximab resulted in full occupancy of BTK at both timepoints assessed (ie, after 7 and 14 days of treatment; appendix p 24). To explore the effects of this treatment on CLL cell migration in vivo, we tested serial blood CLL samples in chemotaxis assays. The combination of ibrutinib and rituximab significantly reduced CLL cell chemotaxis towards CXCL12 and CXCL13 (appendix p 24). For example, the mean relative numbers of CLL cells from six patients migrating toward CXCL13 decreased from 1906 (SE 556) before therapy to 666 (133) after 14 days, and 1109 (401) after 28 days of treatment.

We assayed serial plasma samples for changes in cytokines and chemokines at earlier timepoints (after 14 and 28 days) and at a later timepoint (after 90 days). We

recorded significant reductions in plasma concentrations of several cytokines that are either derived from CLL cells (CCL3 and CCL4) or originate from cells in the microenvironment (CXCL13). For example, after 14 days and 28 days of ibrutinib and rituximab treatment, raised median plasma concentrations of CCL3 decreased significantly from 139·6 (SE 40·4) to 6·9 (0·9) pg/mL, and concentrations of CCL4 fell from 578·6 (SE 103·9) to 68·7 (7·2) pg/mL (appendix p 24). Plasma concentrations of CCL19 TNFα (appendix p 24) and CCL21 (data not shown) were also significantly reduced after 1 month. CXCL13 plasma concentrations decreased significantly from 94·6 (SE 16·3) pg/mL to 32·26 (6·9) pg/mL after 1 month and to 28·7 (SE 6·8) pg/mL after 3 months of ibrutinib and rituximab. Concentrations of CXCL12 remained stable during treatment (appendix p 24).

Discussion

The combination of ibrutinib and rituximab induced responses in a large proportion of patients with high-risk CLL. Responses were durable, and 78% of patients were free of disease progression and 84% still alive at 18 months.

Although cross-trial comparisons have obvious limitations (panel), one of the most notable findings in this study is the short duration of ibrutinib-associated redistribution lymphocytosis. Fewer patients had persistent lymphocytosis than with single-agent treatment,[2] even at early timepoints, presumably caused by the addition of rituximab. 20% of patients treated with 420 mg ibrutinib daily as single agent in other trials had persistent lymphocytosis at 12 months,[2,17] by contrast, persistent lymphocytosis was rare after 6 months of ibrutinib and rituximab (8%), and absent after 12 months. Similar findings of a blunted and shortened duration of lymphocytosis were recently reported for the combination of the PI3Kδ inhibitor idelalisib with rituximab in patients with relapsed CLL.[3] Additionally, compared with the reported single-agent experience, objective remissions were achieved in more patients.[2,15]

The relevance of clearance of CLL cells from the peripheral blood during ibrutinib therapy remains largely unknown. Woyach and colleagues[17] recently published data showing that persistent lymphocytosis during ibrutinib therapy was not associated with an adverse outcome, which suggests that elimination of CLL cells from the peripheral blood—traditionally a key response criterion—might not be a necessary goal of ibrutinib-based treatment. In this study, we assessed the activity of ibrutinib in combination with rituximab. Rituximab, which has very low clinical activity in CLL as a single agent, substantially increases PFS and overall survival when added to fludarabine and cyclophosphamide in previously untreated patients with CLL.[6,23] Its role as combination partner with ibrutinib, beyond effective clearance of CLL cells from the peripheral blood, cannot be further defined in this study and is now being addressed in an ongoing randomised trial of ibrutinib versus the combination of ibrutinib and rituximab (NCT02007044) for patients with relapsed CLL (also including previously untreated patients with high-risk CLL with del[17p] or *TP53* mutation).

In addition to BTK, ibrutinib can also interfere with the activity of other kinases that contain a modifiable cysteine residue homologous to Cys-481 in BTK, such as interleukin-2 inducible tyrosine kinase.[27] This kinase is expressed in natural killer and T cells, and Kohrt and colleagues[28] recently reported that ibrutinib antagonises rituximab's anti-lymphoma activity in a mouse model, based on ibrutinib's inhibition of FcR-stimulated natural killer cell function—specifically antibody-dependent cell-mediated cytotoxicity. However, the relative contribution and importance of natural killer cells, monocytes, or other effector cells for CD20 antibody-mediated killing of B cells remains controversial. Other mechanisms of rituximab-induced toxicity towards malignant B cells, which are independent of natural killer cell function (through induction of direct cell death and complement-dependent cytotoxicity) have been documented.[29] The rapid clearance of CLL cells from the peripheral blood in this trial suggests that any antagonistic activity of ibrutinib does not have a significant role in bloodstream clearance of CLL cells. Beyond this idea, to define whether the addition of rituximab affects PFS or overall survival, we will need to wait for mature data from the randomised trial of ibrutinib versus ibrutinib and rituximab. If PFS or overall survival are prolonged with the addition of rituximab, this would suggest an absence of ibrutinib–rituximab antagonism in CLL in human beings.

The PFS and overall survival outcomes of our study compare favourably with other treatment options for similarly high-risk patients with CLL, especially in the relapsed disease setting (panel). In a retrospective single-centre analysis of 174 patients, median PFS and overall survival in affected patients with del(17p) was higher with ibrutinib than with any other treatment,[30] and the high proportion of responses recorded in this study supports the conclusion that, of the approved therapies, ibrutinib alone or in combination with rituximab is a highly effective treatment for high-risk CLL. Besides PFS and overall survival, improvement in quality of life has become one of the major endpoints in clinical trials of novel anticancer drugs, especially in incurable diseases. CLL has a major effect on quality of life, which is especially poor in older patients, those with advanced-stage disease, fatigue, co-morbidity, and those who are receiving active treatment.[31] Eichhorst and colleagues[32] reported moderate improvement in quality of life after treatment with fludarabine and cyclophosphamide. Our finding of a significant improvement in quality of life during treatment with ibrutinib and rituximab supports the notion that effective treatment of CLL improves quality of life.

The gene-expression profile data (appendix p 23) show on-target downregulation of B-cell receptor-regulated and NFκB-regulated genes that are believed to be central for CLL cell survival and interactions within the lymphoid

tissue microenvironment.[33] For example, the chemokine CCL3, which is upregulated in CLL cells after nurse-like cell co-culture and after B-cell receptor triggering,[34] can attract accessory cells, such as T cells and monocytes, into the lymphoid tissues, fostering the influx of cells that support the survival and growth of the CLL clone. Leukaemia cells isolated from CLL lymph nodes contain high amounts of CCL3,[33] and high CCL3 plasma concentrations are predictive of a poor outcome in this cancer.[35] Importantly, down-modulation of CCL3 during ibrutinib and rituximab combination therapy occurred at the gene expression level and in patients' plasma samples, which serves as a cross-validation of the data and corroborates the value of CCL3 as a plasma biomarker for responses to kinase inhibitors that target B-cell receptor signalling, such as ibrutinib[19] and idelalisib.[36]

The biologically most interesting correlative findings in this study are the normalisation of high peripheral blood T-cell counts and of the concentrations of several cytokines, chemokines, and β_2-microglobulin. T-cell numbers of both the CD4 and CD8 subsets are increased in untreated patients with CLL, and the normalisation of these cell populations during ibrutinib and rituximab therapy (figure 3) can be interpreted as a sign of co-evolution and interdependence between T cells and leukaemia cells. Significant reductions in plasma levels of TNFα, the lymph node-homing chemokines CCL19 and CXCL13, and β_2-microglobulin (appendix p 24) provide further insight into the targets and consequences of ibrutinib and rituximab therapy. Together with the inhibition of chemotaxis of CLL cells isolated from patients on this combination treatment (appendix p 24), which was expected from preclinical work[19,20] and which is due to the direct effects of BTK inhibition on chemokine receptor signalling,[19,20] the decrease in CCL19 and CXCL13 plasma levels (appendix p 24) points towards additional mechanisms for redistribution of tissue leukaemia cells into the peripheral blood during ibrutinib therapy. CCL19 and CXCL13 are important chemokines that coordinate normal and malignant B-cell homing into secondary lymphoid tissues,[37] and their downmodulation during ibrutinib and rituximab combination therapy could be an additional, indirect mechanism for CLL cell mobilisation by ibrutinib. Significant reductions of raised TNFα and β_2-microglobulin concentrations support the important role of these proteins in disease progression and prognosis, but no data exist to suggest a mechanism of regulation of these proteins by the combination of ibrutinib and rituximab.

The main strength of our study is a comprehensive set of clinical and correlative data that show safety and exceptionally high rates of responses with the combination of ibrutinib and rituximab in patients with CLL who have high-risk disease features. These findings are of great interest for this difficult-to-treat patient population, which is in particular need of new treatment options. However, because of the non-randomised approach in this phase 2 trial, we cannot make any definitive conclusions about the added benefit of rituximab. Hence, a general recommendation for use of ibrutinib in combination with rituximab cannot be made at this time, until data from a randomised trial (ibrutinib versus ibrutinib plus rituximab) are available. The correlative data show on-target down-modulation of BCR-regulated genes (eg, CCL3), normalisation of raised T cell numbers and other markers of CLL disease activity (eg, β_2-microglobulin), and stable immunoglobulin levels (except for IgM levels, which trended downwards). These correlative findings provide a fascinating insight into regulatory circuits that govern CLL disease biology, and how these circuits are thwarted by targeted treatment. Several of these mechanisms probably also apply to other related mature B-cell malignancies, in which ibrutinib therapy, alone or in combinations, is currently being explored.

In summary, our findings show that the combination of ibrutinib and rituximab is an active therapy for patients with high-risk CLL, in whom it induces a high proportion of objective responses and durable remissions in most patients. Treatment is well tolerated and is associated with significant improvements in quality of life. A randomised study of ibrutinib versus ibrutinib plus rituximab is ongoing (NCT02007044) and will establish whether the addition of rituximab, besides abridging the initial lymphocytosis, improves PFS, overall survival, or both.

Panel: Research in context

Systematic review

We searched PubMed for articles published in English since Jan 1, 2005, until April 30, 2014, to identify studies of drugs used to treat patients with high-risk CLL (alemtuzumab, chlorambucil, rituximab, ofatumumab, obinutuzumab, fludarabine, cyclophosphamide, and lenalidomide). We identified primary publications for these agents[6,8,23,24] that show that low proportions of patients respond to treatment and short remissions are a major problem. A number of trials of single-agent ibrutinib trials have been published.[12,15,25,26] To our knowledge, no other data for ibrutinib combination therapy are yet available.

Interpretation

Our data suggest that the combination of ibrutinib and rituximab is active and safe for the treatment of patients with high-risk CLL. A randomised study of ibrutinib versus ibrutinib plus rituximab is ongoing (NCT02007044) and will establish whether the addition of rituximab, improves survival outcomes for patients with CLL. This ongoing trial is for patients with CLL with relapsed disease, but also includes previously untreated patients with high-risk CLL with del(17p) or *TP53* mutation.

Contributors

JAB designed and supervised the trial and correlative studies, analysed the data, and wrote the report. MJK, AF, HK, WGW, and SOB contributed to the trial design, clinical patient management, sample collection, and clinical data analysis, and reviewed and approved the report. GZ is the research nurse and JLJ is the pathologist who analysed all the flow data for MRD. EH and AR analysed gene-expression profiles. JH, NYR, and IDW analysed correlative samples. SL, GJ, MC-T, GMN-G, and XH did statistical analyses of the data, and NG analysed serial imaging studies.

Declaration of interests

JAB and SOB received research funding from Pharmacyclics Inc. JAB is a consultant for Janssen Pharmaceuticals Inc. All other authors declare no competing interests.

Acknowledgments

This study was supported by Pharmacyclics Inc, Cancer Prevention and Research Institute of Texas, Leukemia & Lymphoma Society, the National Cancer Institute (grant P30 CA016672), MD Anderson's Moon Shot Program in Chronic Lymphocystic Leukaemia, and MD Anderson Cancer Center support grant CA016672. We thank the patients who participated in this trial and their families, and the study investigators and coordinators at MD Anderson Cancer Center, especially Benjamin Hayes for sample and data collection and Jeannice Theriot for expert editorial assistance.

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