Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial

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Summary

Background On the basis of promising results that were reported in several phase 2 trials, we investigated whether the addition of the monoclonal antibody rituximab to first-line chemotherapy with fludarabine and cyclophosphamide would improve the outcome of patients with chronic lymphocytic leukaemia.

Methods Treatment-naive, physically fit patients (aged 30–81 years) with CD20-positive chronic lymphocytic leukaemia were randomly assigned in a one-to-one ratio to receive six courses of intravenous fludarabine (25 mg/m^2 per day) and cyclophosphamide (250 mg/m^2 per day) for the first 3 days of each 28-day treatment course with or without rituximab (375 mg/m^2 on day 0 of first course, and 500 mg/m^2 on day 1 of second to sixth courses) in 190 centres in 11 countries. Investigators and patients were not masked to the computer-generated treatment assignment. The primary endpoint was progression-free survival (PFS). Analysis was by intention to treat. This study is registered with ClinicalTrials.gov, number NCT00281918.

Findings 408 patients were assigned to fludarabine, cyclophosphamide, and rituximab (chemoimmunotherapy group) and 409 to fludarabine and cyclophosphamide (chemotherapy group); all patients were analysed. At 3 years after randomisation, 65% of patients in the chemoimmunotherapy group were free of progression compared with 45% in the chemotherapy group (hazard ratio 0·56 [95% CI 0·46–0·69], p<0·0001); 87% were alive versus 83%, respectively (0·67 [0·48–0·92]; p=0·01). Chemoimmunotherapy was more frequently associated with grade 3 and 4 neutropenia (136 [34%] of 404 *vs* 83 [21%] of 396; p<0·0001) and leucocytopenia (97 [24%] *vs* 48 [12%]; p<0·0001). Other side-effects, including severe infections, were not increased. There were eight (2%) treatment-related deaths in the chemoimmunotherapy group compared with ten (3%) in the chemotherapy group.

Interpretation Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab improves progression-free survival and overall survival in patients with chronic lymphocytic leukaemia. Moreover, the results suggest that the choice of a specific first-line treatment changes the natural course of chronic lymphocytic leukaemia.

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Introduction

Chronic lymphocytic leukaemia is the most common adult lymphoid malignant disease in western countries, affecting about five in 100 000 of the population per year.[1,2] Its clinical course is highly variable and can be predicted by use of various criteria,[3] including clinical staging,[4,5] chromosomal abnormalities,[6] or mutations of the immunoglobulin heavy variable chain (*IGHV*) gene.[7,8]

For more than 40 years, chronic lymphocytic leukaemia has been treated with various chemotherapies. Chlorambucil, an alkylating drug, was the main drug for three decades.[9,10] Combinations of alkylating drugs with vinca alkaloids or anthracycline drugs did not improve outcomes.[11] In the 1990s, purine analogues were a new class of drugs that were active against chronic lymphocytic leukaemia.[12,13] In the past decade, the combination of purine analogues with alkylating drugs, particularly fludarabine and cyclophosphamide, improved the rates of clinical response, complete remission, and progression-free survival (PFS).[14–16] Until now, however, none of these drug combinations have shown an improvement in overall survival in clinical studies.

Chemoimmunotherapy—the addition of monoclonal antibodies to chemotherapy—was created for the treatment of indolent and aggressive lymphoma. Rituximab, a chimeric monoclonal antibody directed against the CD20 antigen,[17] became the standard drug for use with chemotherapy for various B-cell lymphomas.[18,19] In chronic lymphocytic leukaemia, however, the low expression of the CD20 antigen on leukaemic cells,[20,21] and poor response rates with standard-dose rituximab led to the initial expectation that rituximab might not generate sufficient clinical benefit in this disease.[22] However, higher doses of

rituximab used alone improved response rates.[23] Moreover, the results of phase 2 trials suggested that rituximab in combination with chemotherapy might have additive or synergistic effects in pretreated and treatment-naive patients.[24–28] In these trials, the standard dose of rituximab (375 mg/m² per day) was increased to 500 mg/m² per day.[24] In a study of 300 treatment-naive patients, the combination of fludarabine, cyclophosphamide, and rituximab resulted in an overall response rate of 95%, with 72% of patients achieving a complete response.[24,28] These response rates were among the highest reported so far for first-line treatments in patients with chronic lymphocytic leukaemia. On the basis of these promising results, the German Chronic Lymphocytic Leukaemia Study Group initiated a phase 3 study to compare the efficacy and safety of fludarabine and cyclophosphamide with fludarabine, cyclophosphamide, and rituximab as first-line treatment in patients with advanced, symptomatic chronic lymphocytic leukaemia.

Methods

Study design and patients

A prospective, randomised, open-label, phase 3 study was done in 190 centres in 11 countries. Treatment-naive patients (aged 30–81 years) diagnosed with immunophenotypically confirmed chronic lymphocytic leukaemia in Binet stage C,[4] or with confirmed active disease in Binet stages A or B[29] were enrolled (webappendix p 1). Additional inclusion criteria were an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 and a low comorbidity, which was defined as a cumulative illness rating scale[30] of up to 6, and a creatinine clearance of at least 1·17 mL/s (webappendix p 1). The main exclusion criteria were the absence of active disease in patients with Binet stage A or B, and the presence of clinically apparent autoimmune cytopenia or active second disease.

The institutional review board or ethics committee of each institution approved the study protocol. Each patient provided written informed consent before enrolment.

Randomisation and masking

After informed consent was obtained from the patients, investigators faxed the required registration sheets to the central study office in Cologne, Germany. Patients were randomly assigned in a one-to-one ratio in a block size of four to receive fludarabine and cyclophosphamide (chemotherapy) or fludarabine, cyclophosphamide, and rituximab (chemoimmunotherapy) up to July 27, 2004, stratified by centre, and then by country and Binet stage since the first amendment (July 27, 2004), using a randomisation list that was computer generated at the Institute for Medical Statistics and Epidemiology, Technical University of Munich, Germany. Confirmation of patient randomisation and allocation to treatment group was sent through the Central Study Office in Cologne, Germany, to the investigators. Patients were enrolled by the investigators, and assignment to treatment was done centrally at the Institute for Medical Statistics and Epidemiology, Technical University of Munich. Thus, patient allocation was done independently of the study investigators. Investigators and patients were not masked to the treatment assignment.

Procedures

Treatment consisted of six 28-day courses of intravenous fludarabine (25 mg/m² per day) and cyclophosphamide (250 mg/m² per day) for the first 3 days of each treatment course, with or without rituximab (Mabthera or Rituxan, Roche, Welwyn Garden City, UK) at a dose of 375 mg/m² on day 0 of the first course, and 500 mg/m² on day 1 of the second to sixth courses. Prophylaxis with antiviral drugs or granulocyte-colony stimulating factor were not recommended in this study (webappendix p 1). Prophylaxis of pneumonia caused by *Pneumocystis jirovecii* was recommended for severe leucocytopenia that lasted for more than 7 days.

An interim response assessment was done after three courses of treatment. Patients who achieved at least a partial response or complete remission continued treatment as planned in the protocol. Patients with stable or progressive disease discontinued study

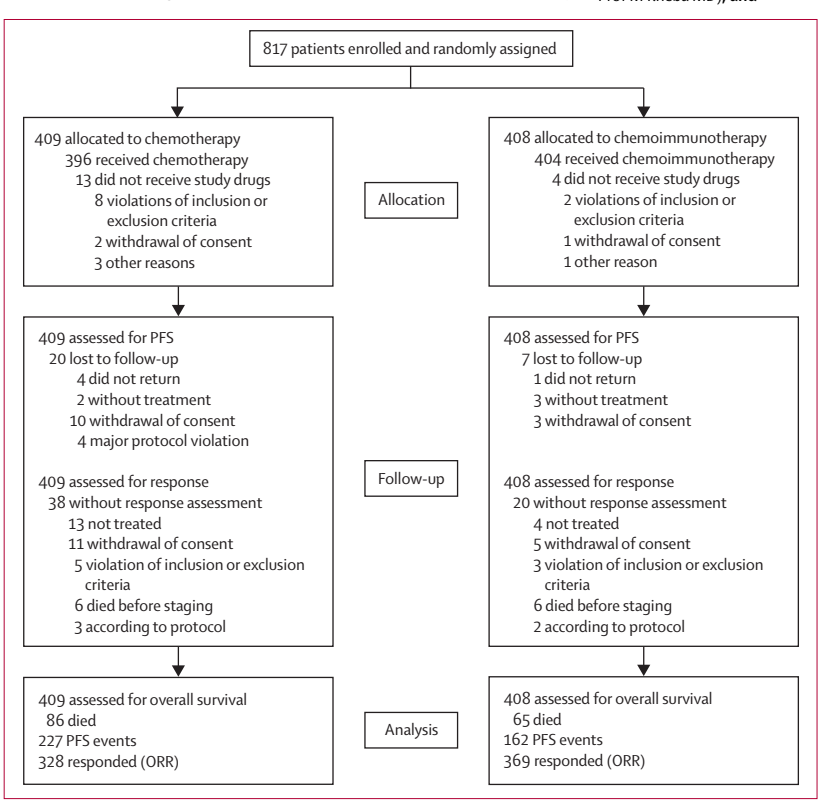

Figure 1: **Trial profile**
Chemotherapy=fludarabine and cyclophosphamide. Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. PFS=progression-free survival. ORR=overall response rate.

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See Online for webappendix

treatment and received a different treatment at the discretion of their treating physician. Patients with stable disease or progressive disease after three cycles were assessed as non-responders and included in the analysis of PFS and overall survival.

An assessment of initial response after completion of treatment was done at about 1 month (plus or minus 7 days) after the start of the previous course of treatment. The results were confirmed at the final assessment that was done at least 2 months later. Subsequently, patients completed follow-up examinations every 3 months for the following years 1–3, every 6 months for years 4–5, and every year up to year 8.

Assessments of clinical disease were completed at baseline, including assessments of tumour, cumulative illness rating scale, ECOG performance status, and immunophenotyping of circulating lymphocytes. Analyses of genomic aberrations by use of fluorescent in-situ hybridisation (FISH),[6] and *IGHV* mutation status[7,8] by use of DNA sequencing were done in the central reference laboratory (Ulm, Germany).

Response to treatment and disease progression were classified according to the National Cancer Institute's working group criteria of 1996.[29] Responses and disease progression were assessed by the study investigators and verified by a central, investigator-independent medical review. Adverse events and serious adverse events were documented according to the Common Toxicity Criteria (version 2).

In January, 2008, the preplanned interim analysis showed a significant difference in the primary efficacy analysis (PFS) in favour of the group assigned to chemoimmunotherapy. Since the significance exceeded the threshold for early closure, the study was formally ended after a recommendation by the data safety monitoring board. At this point, all patients had been enrolled and completed treatment.

	Chemotherapy	Chemoimmunotherapy
Age (years; median, range)	61 (36–81)	61 (30–80)
Age (years)		
≥65	119/409 (29%)	126/408 (31%)
≥70	37/409 (9%)	44/408 (11%)
Men	304/409 (74%)	303/408 (74%)
Binet stage		
A	22/409 (5%)	18/408 (4%)
B	259/409 (63%)	263/408 (64%)
C	126/409 (31%)	126/408 (31%)
ECOG 0	226/390 (58%)	221/395 (56%)
Presence of B symptoms	197/406 (49%)	167/407 (41%)
Cumulative illness rating scale (median, range)	1 (0–8)	1 (0–7)
CD20-positive cells by flow cytometry (median, range)	81% (0–100)	79% (0–100)
Creatinine clearance <1·17 mL/s	88/392 (22%)	94/398 (24%)
IGHV unmutated	194/310 (63%)	196/309 (63%)
Cytogenetic abnormalities		
Del(13q)	182/305 (60%)	168/312 (54%)
Del(11q)	69/307 (22%)	84/314 (27%)
Trisomy 12	44/306 (14%)	30/312 (10%)
Del(17p)	29/306 (10%)	22/315 (7%)
β_2 microglobulin (≥3·5 mg/L)	85/266 (32%)	91/277 (33%)
Serum thymidine kinase (≥10 U/L)	206/266 (77%)	202/277 (73%)
ZAP70 expression	55/147 (37%)	59/142 (42%)
Positive CD38	239/359 (67%)	237/360 (66%)

Data are n/N (%), unless otherwise indicated. Chemotherapy=fludarabine and cyclophosphamide. Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. ECOG=Eastern Cooperative Oncology Group.

Table 1: **Patients' demographics and baseline characteristics**

Statistical analysis

The primary endpoint was PFS, defined as the time between randomisation and the date of first documented disease progression or death from any cause. Secondary endpoints were event-free survival; overall survival; disease-free survival; duration of remission; time to new treatment for chronic lymphocytic leukaemia or death; rates of molecular, complete, and partial remission; response rates and survival times in biological subgroups; rates of treatment-related adverse effects; pharmaco-economic effect; and quality of life. PFS and results of safety and the major secondary endpoints (overall survival and response rates) for the entire study cohort and biological subgroups are reported here (figure 1).

The planned sample size was calculated on the basis of the primary endpoint, PFS. Data that were reported before the initiation of the study indicated that the median PFS was expected to be 40 months in the chemotherapy group (corresponding to a 66% PFS rate at 2 years), and 54 months in the chemoimmunotherapy group (corresponding to a 74% PFS rate at 2 years).[15] On the assumption that the hazard ratio (HR) was 0·741, using a two-sided log-rank test at the 5% level with a power of 80%, and accounting for one interim analysis, a minimum of 357 events were required. The interim analysis was done after two-thirds of the required number of events—ie, 238 events, had occurred. Exploratory analyses of the secondary endpoints were also done. To confirm the primary analysis, a two-sided log-rank test stratified by Binet stage was done before treatment. Exploratory analyses for the secondary endpoints were done with two-sided stratified and non-stratified log-rank tests for time-to-event outcomes, Fisher's exact test and χ^2 test for categorical variables, and Mann-Whitney tests for continuous variables. A Cox proportional hazard model with stepwise backward selection was applied to PFS and overall survival, including treatment and prognostic factors—type of treatment, age, sex, disease stage, time from first diagnosis, ECOG performance status, presence of B symptoms (night sweats, weight loss ≥10% within the previous 6 months, fever >38°C or 100·4°F for ≥2 weeks without evidence of infection), white blood cell count, serum thymidine kinase and β_2 microglobulin

concentrations, specific cytogenetic aberrations,[6] and *IGHV* mutational status. Cutoff concentrations of continuous variables were used as previously reported.[31] Optimum cutoff concentrations were 10 U/L for serum thymidine kinase and 3·5 mg/L for β_2 microglobulin. The two-sided significance level was set at 0·05. Analysis was by intention to treat.

This study is registered with ClinicalTrials.gov, number NCT00281918.

Role of the funding source

This trial was planned and initiated in 2003 as an investigator-initiated trial by the German Chronic Lymphocytic Leukaemia Study Group. Since 2004, F Hoffmann-La Roche assumed the sponsorship for this trial, because it intended to use the trial for the approval of rituximab at regulatory agencies. The sponsor was subsequently involved in the first and second amendments of the study protocol. The sponsor of the study was responsible for data gathering, and shared responsibility for medical review of the data with the corresponding author. The corresponding author was responsible for data analysis, data interpretation, writing of the report, had full access to all the data in the study, and had the final responsibility for the decision to submit for publication.

Results

Previously untreated patients with chronic lymphocytic leukaemia were enrolled between July, 2003, and March, 2006. Figure 1 shows the trial profile. Patients were randomly assigned to receive chemotherapy with fludarabine and cyclophosphamide, or chemo-immunotherapy with fludarabine, cyclophosphamide, and rituximab. Both groups were well balanced with respect to age, sex, disease stage, physical fitness (cumulative illness rating scale, ECOG), creatinine clearance, serum concentrations of β_2 microglobulin and thymidine kinase, genomic aberrations, and *IGHV* mutational status (table 1). Patients in the chemotherapy group had B symptoms more often than did those in the chemoimmunotherapy group. Genomic profiling data (FISH and *IGHV* mutational status) were available for 624 (76%) patients. This cohort was representative of the full trial population with respect to demographic characteristics and other baseline prognostic factors.

The mean number of treatment courses administered was 5·2 (range 0–6) in the chemoimmunotherapy group compared with 4·8 (0–6; p=0·006) in patients assigned to chemotherapy. Of the patients receiving the study treatment, 106 (26%) did not receive the planned six courses in the chemoimmunotherapy group compared with 136 (34%) in the chemotherapy group. The higher adherence to treatment in the chemoimmunotherapy group was attributable to the fewer non-responders withdrawn from the study. The median cumulative doses were 778·2 mg for fludarabine and 7735 mg for cyclophosphamide in the chemotherapy group, and 774·0 mg for fludarabine and 7650 mg for cyclophosphamide in the chemoimmunotherapy group

	Chemotherapy	Chemoimmunotherapy	p value
All (n=817)			
CR	88/409 (22%)	180/408 (44%)	<0·0001
ORR	328/409 (80%)	369/408 (90%)	<0·0001
Binet stage A (n=40)			
CR	6/22 (27%)	13/18 (72%)	0·010
ORR	15/22 (68%)	18/18 (100%)	0·11
Binet stage B (n=522)			
CR	66/259 (25%)	124/263 (47%)	<0·0001
ORR	220/259 (85%)	245/263 (93%)	0·003
Binet stage C (n=252)			
CR	16/126 (13%)	43/126 (34%)	<0·0001
ORR	92/126 (73%)	106/126 (84%)	0·04
Patients with cytogenetic results and response assessment (n=623)*			
CR	59/307 (19%)	138/316 (44%)	<0·0001
ORR	243/307 (79%)	290/316 (92%)	<0·0001
Age <65 years (n=572)			
CR	59/290 (20%)	126/282 (45%)	<0·0001
ORR	229/290 (79%)	252/282 (89%)	0·001
Age ≥65 years (n=245)			
CR	29/119 (24%)	54/126 (43%)	0·003
ORR	99/119 (83%)	117/126 (93%)	0·028
Del(17p) (n=51)			
CR	0/29	1/22 (5%)	0·43
ORR	10/29 (34%)	15/22 (68%)	0·025
Del(11q) (n=142)†			
CR	9/62 (15%)	41/80 (51%)	<0·0001
ORR	54/62 (87%)	74/80 (93%)	0·40
Trisomy 12 (n=61)‡			
CR	7/37 (19%)	17/24 (71%)	0·0001
ORR	31/37 (84%)	24/24 (100%)	0·07
Del(13q) (n=224)§			
CR	27/119 (23%)	50/105 (48%)	0·0001
ORR	95/119 (80%)	101/105 (96%)	0·0002
No abnormalities according to the hierarchical model (n=138)¶			
CR	16/58 (28%)	28/80 (35%)	0·5
ORR	53/58 (91%)	71/80 (89%)	0·78
IGHV mutated (n=229)			
CR	24/116 (21%)	56/113 (50%)	<0·0001
ORR	98/116 (84%)	105/113 (93%)	0·06
IGHV unmutated (n=390)			
CR	36/194 (19%)	79/196 (40%)	<0·0001
ORR	148/194 (76%)	179/196 (91%)	<0·0001

Data are n/N (%), unless otherwise indicated. Chemotherapy=fludarabine and cyclophosphamide. Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. CR=complete remission. ORR=overall response rate. *Including seven patients with cytogenetic results outside the hierarchical model. †Not including del(17p). ‡Not including del(17p) or del(11q). §Not including del(17p), del(11q), or trisomy 12. ¶Not including del(17p), del(11q), trisomy 12, or del(13q) (ie, genetic classification according to the hierarchical model6).

Table 2: **Response to treatment in prognostic subgroups**

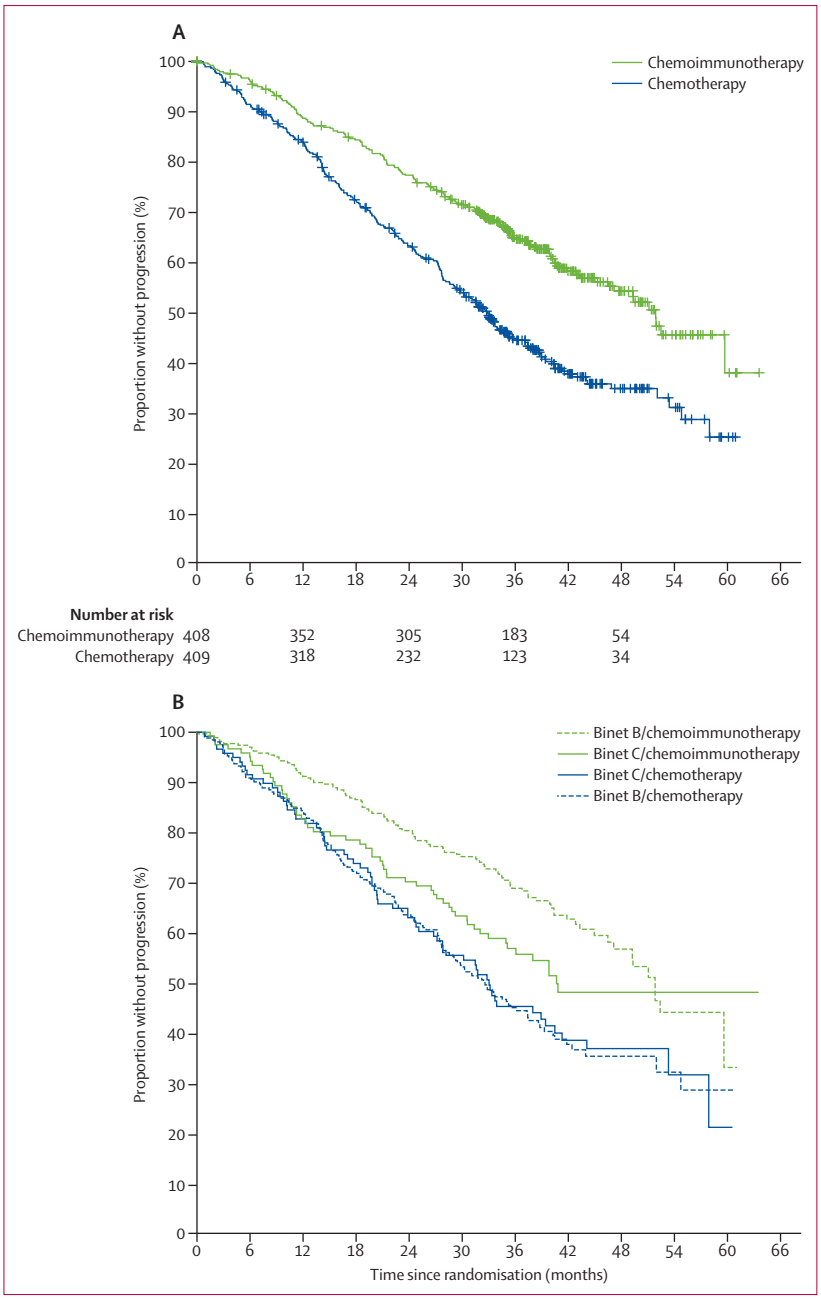

Figure 2: **Progression-free survival in all patients (A) and in patients with Binet stage B and C chronic lymphocytic leukaemia (B)**
Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. Chemotherapy=fludarabine and cyclophosphamide.

(fludarabine, p=0·6; cyclophosphamide, p=0·8). Patients in Binet stages A and B received more treatment courses (mean 5·28 [range 0–6]) than did those in Binet stage C (4·52 [0–6]; p<0·0001). For any of the three drugs, the planned dose was reduced by more than 10% in 189 (47%) of 404 patients in the chemoimmunotherapy group and 108 (27%) of 396 patients in the chemotherapy group (p<0·0001). 207 of 800 patients had dose reductions (>10%) during the first to third courses (chemotherapy 74 [19%] of 396; chemoimmunotherapy 133 [33%] of 404; p<0·0001), and dose reductions occurred in 216 of 800 patients during the fourth to sixth courses (chemotherapy 79 [20%] of 396; chemoimmunotherapy 137 [34%] of 404; p<0·0001). These dose reductions were mostly because of treatment-related haematological toxicity, particularly neutropenia and leucocytopenia (117 [62%] of 189 patients in the chemoimmunotherapy group *vs* 69 [64%] of 108 in the chemotherapy group; webappendix p 2).

Significantly more patients were in complete remission in the chemoimmunotherapy group than in the chemotherapy group (table 2). More patients responded to treatment and more achieved a complete remission in all Binet stages (table 2). The proportion of patients who did not respond to treatment was lower in the chemoimmunotherapy group than in the chemotherapy group (39 [10%] *vs* 81 [20%]; p<0·0001).

PFS was longer in the chemoimmunotherapy group than in the chemotherapy group (median 51·8 months [95% CI 46·2–57·6] *vs* 32·8 months [29·6–36·0]; p<0·0001; figure 2A). At 3 years after randomisation, more patients remained progression free in the chemoimmunotherapy group than in the chemotherapy group (figure 2A; table 3). The risk of progression was reduced by 44% in the chemoimmunotherapy group compared with the chemotherapy group (HR 0·56 [95% CI 0·46–0·69]). An improvement in PFS was noted in all stages. Patients with disease in Binet stages B and C showed similar median PFS of 32·5 months (28·4–36·6) and 33·0 months (25·0–41·2), respectively, when treated with chemotherapy (figure 2B). Treatment with chemoimmunotherapy improved the median PFS to 51·8 months (47·8–56·0) in 522 patients with Binet stage B disease (HR 0·50 [95% CI 0·39–0·65]; p<0·0001) and to 40·7 months (0·73 [0·51–1·04]; p=0·081) in 252 patients with Binet stage C disease (figure 2B). Patients with Binet stage C disease who were given chemoimmunotherapy compared with those who were given chemotherapy showed an accumulation of several unfavourable factors (all p>0·05): age 65 years or older (44 [35%] of 126 *vs* 33 [26%] of 126), unmutated *IGHV* status (53 [54%] of 99 *vs* 45 [48%] of 94), elevated ZAP70 concentrations (19 [42%] of 45 *vs* 15 [33%] of 45), and β_2 microglobulin concentrations greater than 3·5 mg/L (47 [52%] of 91 *vs* 37 [44%] of 84). Dose reductions of more than 10% were more common in patients with Binet stage C disease in the chemoimmunotherapy group (34 [28%] of 122 *vs* 61 [49%] of 124; p=0·001), mostly because of neutropenia and leucocytopenia (21 [55%] of 38 *vs* 42 [62%] of 68). At 3 years after randomisation, fewer patients with Binet stage B disease and Binet stage C disease in the chemotherapy group than in the chemoimmunotherapy group were progression free (table 3). The small number of patients (n=40) in Binet stage A did not allow a meaningful analysis of this subgroup, but a non-significant

improvement was noted in PFS with chemoimmunotherapy.

Fludarabine, cyclophosphamide, and rituximab also resulted in a significant benefit with respect to overall survival. More deaths were noted after treatment with fludarabine and cyclophosphamide (86 [21%] of 409) than after treatment with fludarabine, cyclophosphamide, and rituximab (65 [16%] of 408). In most cases, the underlying cause of death was progressive disease (chemotherapy 48 [56%] of 86, chemoimmunotherapy 33 [51%] of 65). Other fatal events were secondary cancers (chemotherapy 13 [15%], chemoimmunotherapy five [8%]) and causes unrelated to chronic lymphocytic leukaemia, such as myocardial infarction (15 [17%] and 17 [26%], respectively). At 3 years after randomisation, more patients in the chemoimmunotherapy group were alive than in the chemotherapy group (table 4). The time to 25% of patients dying was 62·5 months in the chemoimmunotherapy group and 46·8 months in the chemotherapy group (p=0·012; figure 3A). The risk of death was reduced by 33% in the chemoimmunotherapy group (n=355) compared with the chemotherapy group (HR 0·67 [95% CI 0·48–0·92], p=0·012).

The benefit of chemoimmunotherapy was noted in patients younger than 65 years and in those 65 years or older with respect to the response rates and time to progression, but not survival time (tables 2–4). Moreover, in Cox regression analyses, age was not an independent predictor of PFS or overall survival (table 5). Analyses of secondary endpoints such as event-free survival, duration of remission, and time to new treatment confirmed the clinical benefit with chemoimmunotherapy (webappendix p 1).

More patients in the chemoimmunotherapy group had a complete remission in most genetic subgroups as defined by use of the hierarchical model,[6] except for patients with del(17p) or with no abnormalities (table 2). In patients with del(11q), trisomy 12, and unmutated *IGHV*, chemoimmunotherapy improved rates of complete remission by 3·5, 3·7, and 2·2 times, respectively (table 2). At 3 years after randomisation, 76% of 268 patients who achieved a complete remission remained progression free compared with 45% of 549 who achieved only a partial response or a non-response (p<0·0001); 95% of 268 patients who achieved complete remission were alive compared with 82% of 549 who achieved a partial response or non-response (p<0·0001).

Chemoimmunotherapy resulted in significantly higher PFS in most genetic subgroups, including del(17p), del(11q), del(13q), and trisomy 12 (table 3). The del(17p) subgroup had the shortest median PFS (chemoimmunotherapy 11·3 months [range 10·3–12·2] *vs* chemotherapy 6·5 months [0·8–12·2]; HR 0·47 [95% CI 0·24–0·90]; p=0·019). An unmutated *IGHV* was predictive of a shorter PFS (table 5). Chemoimmunotherapy also improved overall survival in most subgroups of patients, including Binet stages A and B (but not stage C), del(13q), del(11q), and unmutated *IGHV* (table 4).

	Chemotherapy	Chemoimmunotherapy	Hazard rate (95% CI)	p value
All (n=817)	45%	65%	0·56 (0·46–0·69)	<0·0001
Binet stage				
A (n=40)	42%	62%	0·42 (0·16–1·14)	0·08
B (n=522)	45%	69%	0·50 (0·39–0·65)	<0·0001
C (n=252)	45%	57%	0·73 (0·51–1·04)	0·08
Age				
<65 years (n=572)	46%	64%	0·57 (0·45–0·73)	<0·0001
≥65 years (n=245)	43%	68%	0·55 (0·38–0·79)	0·001
Del(17p) (n=51)	0	18%	0·47 (0·24–0·90)	0·019
Del(11q) (n=142)*	32%	64%	0·34 (0·24–0·61)	<0·0001
Trisomy 12 (n=61)†	48%	83%	0·32 (0·13–0·80)	0·01
Del(13q) (n=224)‡	52%	76%	0·43 (0·28–0·68)	0·0002
No abnormalities according to the hierarchical model (n=138)§	48%	58%	0·78 (0·48–1·30)	0·3
IGHV mutated (n=229)	55%	80%	0·43 (0·27–0·69)	0·0002
IGHV unmutated (n=390)	35%	55%	0·62 (0·48–0·81)	0·0003

Data are percentages (Kaplan-Meier estimates), unless otherwise indicated. Chemotherapy=fludarabine and cyclophosphamide. Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. *Not including del(17p). †Not including del(17p) or del(11q). ‡Not including del(17p), del(11q), or trisomy 12. §Not including del(17p), del(11q), trisomy 12, or del(13q) (ie, genetic classification according to the hierarchical model[6]).

Table 3: **Progression-free survival at 3 years after randomisation in prognostic subgroups**

Del(17p), chemotherapy, unmutated *IGHV* status, β_2 microglobulin serum concentrations of at least 3·5 mg/L, and white blood cell count of 50×10^9 per L were predictive of a reduced PFS (table 5). Variables that were predictive of a reduced overall survival were treatment with chemotherapy, del(17p), β_2 microglobulin serum concentrations of at least 3·5 mg/L, serum thymidine kinase concentration of at least 10 U/L, and an ECOG performance status of at least 1.

The presence of del(17p) was the strongest negative prognostic indicator of PFS and overall survival (table 5). Patients with this genetic abnormality showed a significantly shorter overall survival than did those in all the other cytogenetic subgroups (p<0·0001), irrespective of the treatment given (figure 3B and C). Preliminary data about secondline treatment in patients with del(17p) are described in the webappendix p 3.

All patients who were given at least one dose of at least one of the study drugs were included in the safety analysis. The total number of patients who had at least one grade 3 or 4 event during treatment was higher in the chemoimmunotherapy group than in the chemotherapy group (table 6). The frequency of side-effects (eg, thrombocytopenia, anaemia, infections, tumour lysis syndrome, and cytokine release syndrome) was not significantly different in the two groups, with the exception of neutropenia and leucocytopenia, which occurred more often in the chemoimmunotherapy group (table 6).

	Chemotherapy	Chemoimmunotherapy	Hazard rate (95% CI)	p value
All (n=817)	83%	87%	0·67 (0·48–0·92)	0·012
Binet stage				
A (n=40)	84%	94%	0·19 (0·02–1·61)	0·091
B (n=522)	81%	90%	0·45 (0·30–0·69)	0·0002
C (n=252)	85%	81%	1·48 (0·84–2·62)	0·168
Age				
<65 years (n=572)	85%	87%	0·68 (0·46–1·02)	0·059
≥65 years (n=245)	78%	88%	0·63 (0·37–1·10)	0·103
Del(17p) (n=51)	37%	38%	0·66 (0·32–1·36)	0·25
Del(11q) (n=142)*	83%	94%	0·42 (0·18–0·97)	0·036
Trisomy 12 (n=61)†	86%	96%	0·23 (0·03–1·94)	0·142
Del(13q) (n=224)‡	89%	95%	0·30 (0·13–0·71)	0·004
No abnormalities according to the hierarchical model (n=138)§	87%	83%	1·56 (0·67–3·64)	0·303
IGHV mutated (n=229)	89%	91%	0·70 (0·33–1·49)	0·354
IGHV unmutated (n=390)	79%	86%	0·62 (0·41–0·94)	0·023

Data are percentages (Kaplan-Meier estimates), unless otherwise indicated. Chemotherapy=fludarabine and cyclophosphamide. Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. *Not including del(17p). †Not including del(17p) or del(11q). ‡Not including del(17p), del(11q), or trisomy 12. §Not including del(17p), del(11q), trisomy 12, or del(13q) (ie, genetic classification according to the hierarchical model[6]).

Table 4: **Overall survival at 3 years after randomisation in prognostic subgroups**

	Progression-free survival		Overall survival	
	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Chemoimmunotherapy	0·48 (0·37–0·61)	<0·0001	0·61 (0·41–0·91)	0·017
Serum β_2 microglobulin ≥3·5 mg/L	1·40 (1·09–1·81)	0·009	1·82 (1·19–2·79)	0·006
ECOG performance status ≥1	..	..	1·85 (1·23–2·78)	0·003
Serum thymidine kinase ≥10 U/L	..	..	1·87 (1·02–3·41)	0·042
Del(17p)	7·49 (4·83–11·61)	<0·0001	9·32 (5·24–16·56)	<0·0001
IGHV unmutated	1·51 (1·11–2·05)	0·008	..	..
White blood cell count ≥50×10^9 per L	1·41 (1·08–1·86)	0·013	..	..

Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. ECOG=Eastern Cooperative Oncology Group.

Table 5: **Multivariate analysis of the effects of various prognostic variables on progression-free survival and overall survival in 524 patients**

138 (17%) of 800 patients discontinued treatment as a result of adverse events. More patients who were 65 years or older than those who were younger than 65 years had adverse events (table 6). Haematological toxicity was more frequent in patients 65 years or older who were treated with chemoimmunotherapy, without any increase of the total rate of infections. Bacterial infections occurred more frequently in patients 65 years or older than in younger patients (table 6).

Granulocyte-colony stimulating factor was administered in 86 treatment cycles, for a median of 7 days in the chemotherapy group and 6 days in the chemoimmunotherapy group. It was given more frequently in the chemoimmunotherapy group (n=75 *vs* n=11). In 40 treatment cycles, granulocyte-colony stimulating factor was given as prophylaxis without any sign of neutropenia; in 46 cycles, it was administered to treat an adverse event (neutropenia or leucocytopenia).

Ten (3%) deaths were related to treatment in the chemotherapy group, and eight (2%) in the chemoimmunotherapy group. Of these, six chemotherapy-treated patients and five chemoimmunotherapy-treated patients died from infections (septicaemia [n=6], pneumonia [n=3], hepatitis B [n=1], and cryptosporidium gastroenteritis [n=1]). In seven patients (three in the chemotherapy group and four in the chemoimmunotherapy group), death occurred before the third treatment course (fatal septicaemia [n=6], sudden cardiac death [n=1]).

Discussion

The combination of fludarabine, cyclophosphamide, and rituximab substantially increased the proportions of patients achieving a complete remission and remaining free of progression for 3 years. Most importantly, this treatment also increased the likelihood of patients surviving 3 years or more after randomisation (table 4) and was an independent prognostic factor for overall survival in the multivariate analysis. This result is unexpected, because treatment of an indolent disease of recurrent nature such as chronic lymphocytic leukaemia was not thought to require an efficient first-line treatment. Why was a survival benefit not reported in previous trials of patients with chronic lymphocytic leukaemia? First, our trial is one of the largest done so far for first-line treatment of chronic lymphocytic leukaemia. Therefore, the statistical power needed to show effects on survival might not have been achieved in some of the previous studies. Second, the addition of the monoclonal antibody rituximab to chemotherapy was shown to be more effective for treatment of chronic lymphocytic leukaemia than was initially expected.

Improvements in PFS and overall survival were much greater in Binet stages A or B than in stage C, although chemoimmunotherapy also resulted in significantly higher rates of complete remission in patients with Binet stage C disease (table 2). This smaller benefit in Binet stage C might be attributable to a combined imbalance of unfavourable prognostic factors. Since dose reductions for prevention of haematological toxicity needed to be maintained for the rest of the treatment according to the protocol recommendations, we believe that dose reductions required during early courses of chemoimmunotherapy led to persistently lower doses of all three drugs in patients with Binet stage C disease.

B symptoms and trisomy 12 were present slightly more often in the chemotherapy group. To test for potentially relevant imbalances in the study, both these variables were included as potential covariates in the Cox proportional hazard model. Neither contributed

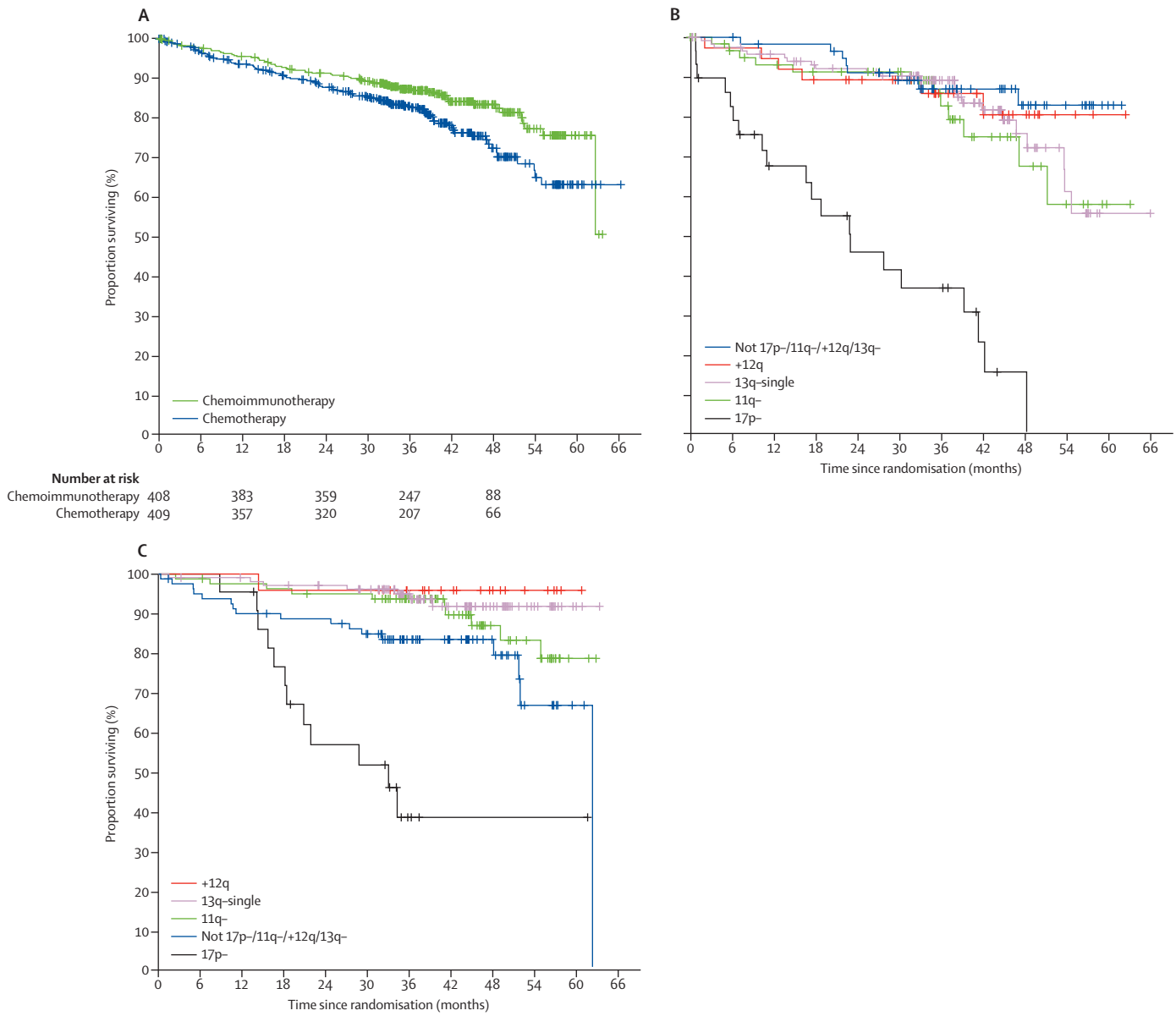

***Figure 3*: Overall survival in all patients (A), and in genetic subgroups of chemotherapy (B) and chemoimmunotherapy groups (C)**
Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab. Chemotherapy=fludarabine and cyclophosphamide.

significantly to the prediction of PFS and OS in this trial.

With the exception of neutropenia and leucocytopenia, the frequency of grade 3 or 4 adverse events, including severe or opportunistic infections, was not increased with fludarabine, cyclophosphamide, and rituximab. Initial reports about the use of rituximab in patients with chronic lymphocytic leukaemia had stressed the possibility of early infusion reactions, cytokine release syndromes, or tumour cell agglutination.[32,33] However, these side-effects did not arise often (<1%) in this trial. Moreover, patients older than 65 years with good physical fitness (defined by use of the cumulative illness rating scale[30] and creatinine clearance) tolerated both treatment regimens quite well, and showed improved outcome and survival time that were similar to those in patients younger than 65 years (tables 2 and 4). However, the patients' median age of 61 years was much younger than that of the average population of patients with chronic lymphocytic leukaemia (with a median age at disease onset of about 70 years). Therefore, the population in this trial represents a selection of fairly young and physically fit patients. As a consequence, conclusions from this trial should not be generalised to

	Chemotherapy (n=396)	Chemoimmunotherapy (n=404)	p value	<65 years (n=560)	≥65 years (n=240)	p value
Total number of patients with at least one grade 3 or 4 event	249 (63%)	309 (76%)	<0·0001	375 (67%)	183 (76%)	0·009
Haematological toxicity	157 (40%)	225 (56%)	<0·0001	254 (45%)	128 (53%)	0·04
Neutropenia	83 (21%)	136 (34%)	<0·0001	146 (26%)	73 (30%)	0·21
Leucocytopenia	48 (12%)	97 (24%)	<0·0001	106 (19%)	39 (16%)	0·37
Thrombocytopenia	44 (11%)	30 (7%)	0·07	50 (9%)	24 (10%)	0·63
Anaemia	27 (7%)	22 (5%)	0·42	35 (6%)	14 (6%)	0·82
Autoimmune haemolytic anaemia	4 (1%)	3 (<1%)	0·69	4 (<1%)	3 (1%)	0·46
Tumour lysis syndrome	2 (<1%)	1 (<1%)	0·55	3 (<1%)	0	0·26
Cytokine release syndrome	0	1 (<1%)	0·32	1 (<1%)	0	0·51
Infections, total	85 (21%)	103 (25%)	0·18	127 (23%)	61 (25%)	0·4
Infections, not specified	68 (17%)	83 (21%)	0·19	104 (19%)	46 (19%)	0·84
Bacterial infection	5 (1%)	11 (3%)	0·14	6 (1%)	10 (4%)	0·004
Viral infection	17 (4%)	17 (4%)	0·95	26 (5%)	8 (3%)	0·4
Fungal infection	1 (<1%)	3 (<1%)	0·33	3 (<1%)	1 (<1%)	0·83
Parasitic infection	0	1 (<1%)	0·32	0	1 (<1%)	0·13

Data are number (%), unless otherwise indicated. Chemotherapy=fludarabine and cyclophosphamide. Chemoimmunotherapy=fludarabine, cyclophosphamide, and rituximab.

Table 6: **Incidence of grade 3 and 4 adverse events**

physically unfit, elderly patients with chronic lymphocytic leukaemia. What remains to be established is how patients given first-line fludarabine and cyclophosphamide, or fludarabine, cyclophosphamide, and rituximab will fare with second-line treatments. Data are being gathered to create a database to address this important question.

The analysis of various clinical and genetic variables showed that the presence of del(17p) was the strongest negative prognostic factor. Del(17p) is associated with a dysfunction of the p53 tumour suppressor[34] and a very poor outcome after fludarabine-based chemotherapies.[34,35] In our trial, the negative prognostic effect of del(17p) was not abrogated with chemoimmunotherapy. By contrast, the rate of complete remission in patients with del(11q), an aberration previously associated with a poor prognosis,[36] was increased by more than three times with chemoimmunotherapy (table 2). Our results corroborate the results of a report showing that this treatment might overcome the adverse prognostic significance of del(11q).[37] Additional subgroups that benefited greatly from treatment with fludarabine, cyclophosphamide, and rituximab were patients with trisomy 12 and del(13q) (table 2).

These results suggest that a molecularly guided treatment approach could be useful for patients with chronic lymphocytic leukaemia; those with del(17p) might not benefit substantially from chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab. Therefore, allogeneic stem cell transplantation should be offered to patients with del(17p) who are physically fit, if they achieve a remission with treatments such as the chemoimmunotherapy we used, alemtuzumab, or similar treatments.[38–40] By contrast, the combination of fludarabine, cyclophosphamide, and rituximab seems an excellent treatment choice for patients with del(11q), trisomy 12, or del(13q). The results of this trial lend support to the recommendation that molecular genetics (FISH) should be used to guide treatment of patients with chronic lymphocytic leukaemia.[41]

Our findings show that the combination of fludarabine, cyclophosphamide, and rituximab as first-line chemoimmunotherapy improves the outcome of physically fit patients with chronic lymphocytic leukaemia. The findings of this randomised trial show an improvement of overall survival after a specific first-line treatment for patients with chronic lymphocytic leukaemia. These results might help establish a new treatment model in which the choice of a specific first-line treatment improves the natural course of chronic lymphocytic leukaemia.

Contributors

MHa, GFR, RB, UJ, CMW, BFE MK, HD, and SS designed the study. MHa and KF co-chaired the study. MHa, KF, MM, GFR, AMF, and AW, were responsible for the conduct of the study. MJ, PLZ, FCC, JFS, ABe, UJ, BC, MT, GHo, GHe, UvG, MB, JC, and MHe were responsible for country-specific issues or supported the conduct of the study. PS, ABü, DW, TZ, SB, MR, MK, HD, and SS did the central laboratory tests, and PS, MK, and SS led the laboratory components of this study. MHa, KF, GFR, RB, AMF, CMW, BFE, SB, MR, MM, MK, HD, and SS analysed the study data. MHa, KF, GFR, RB, and AMF drafted the report, and all coauthors critically revised the report for important intellectual content.

Data Safety and Monitoring Board Members

UK Peter Hillmen (chairman, Leeds Teaching Hospital NHS Trust, Leeds). *France* Guillaume Dighiero (Institut Pasteur, Paris). *Spain* Francesc Bosch (Hospital Clínic, Barcelona). *Italy* Maura Brugiatelli (Azienda Ospedaliera Papardo, Messina). *Germany* Iris Pigeot (Bremen Institute for Prevention Research and Social Medicine Bremen).

Conflicts of interest

MM is a paid employee of Roche. MHa (also from Mundipharma), CMW, JM, JC, JFS, UJ, and SS declared consultancy or board

membership for Roche. GHo declared board membership for Mundipharma. MHa, BFE, JM, GHo (also from Mundipharma), GHe, JC, JFS, UJ, MT, and SS received payment for educational presentations for Roche. GFR, CMW (also from German Cancer Aid), BFE (also from Mundipharma), JM, GHe, MB (also from Celgene and Bayer) JC, JFS (also from Bayer Schering), UJ, BC, MT, PS, TZ, HD, SB, MK, and SS received honoraria or grants from Roche, partly for serving as investigators in Roche-funded research. KF, GFR, AMF, AW, CMW, BFE, JM, MHe, GHe, MB, JC, JFS (also from Bayer Schering), UJ, BC, ABe, MT, PS, DW, TZ, SB, MR, and SS received travel grants from Roche. RB, UvG, PLZ, FCC, and ABü declare that they have no conflicts of interest.

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