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ORIGINAL REPORT

Bendamustine Combined With Rituximab in Patients With Relapsed and/or Refractory Chronic Lymphocytic Leukemia: A Multicenter Phase II Trial of the German Chronic Lymphocytic Leukemia Study Group

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ABSTRACT

Purpose
The objective of this trial was to evaluate safety and efficacy of bendamustine combined with rituximab (BR) in patients with relapsed and/or refractory chronic lymphocytic leukemia (CLL).

Patients and Methods
Seventy-eight patients, including 22 patients with fludarabine-refractory disease (28.2%) and 14 patients (17.9%) with deletion of 17p, received BR chemoimmunotherapy. Bendamustine was administered at a dose of 70 mg/m^2 on days 1 and 2 combined with rituximab 375 mg/m^2 on day 0 of the first course and 500 mg/m^2 on day 1 during subsequent courses for up to six courses.

Results
On the basis of intent-to-treat analysis, the overall response rate was 59.0% (95% CI, 47.3% to 70.0%). Complete response, partial response, and nodular partial response were achieved in 9.0%, 47.4%, and 2.6% of patients, respectively. Overall response rate was 45.5% in fludarabine-refractory patients and 60.5% in fludarabine-sensitive patients. Among genetic subgroups, 92.3% of patients with del(11q), 100% with trisomy 12, 7.1% with del(17p), and 58.7% with unmutated *IGHV* status responded to treatment. After a median follow-up time of 24 months, the median event-free survival was 14.7 months. Severe infections occurred in 12.8% of patients. Grade 3 or 4 neutropenia, thrombocytopenia, and anemia were documented in 23.1%, 28.2%, and 16.6% of patients, respectively.

Conclusion
Chemoimmunotherapy with BR is effective and safe in patients with relapsed CLL and has notable activity in fludarabine-refractory disease. Major but tolerable toxicities were myelosuppression and infections. These promising results encouraged us to initiate a further phase II trial evaluating the BR regimen in patients with previously untreated CLL.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is characterized by its highly variable outcome, with survival after diagnosis ranging from months to decades.[1] To date, allogeneic stem-cell transplantation is the only curative option, but only a few selected patients are considered appropriate candidates for this aggressive treatment modality.[1,2] Nevertheless, over the last decade, considerable progress has been made in the treatment of CLL. The purine analog fludarabine and its combinations have markedly improved the treatment success.[3-8] More recent trials evaluated the impact of chemoimmunotherapy in patients with CLL providing evidence that the addition of the anti-CD20 monoclonal antibody rituximab to fludarabine and cyclophosphamide is highly effective in both relapsed and first-line therapy.[7,9-11] However, almost all patients will eventually experience relapse and may become refractory to fludarabine-containing regimens. These patients have a poor prognosis and usually show only limited response to salvage chemotherapy, with response rates ranging between 22% and 34% and median overall survival times ranging between 10 and 19 months.[12,13]

Bendamustine, an alkylating agent, has been used in Germany for more than 40 years and has

shown considerable activity as a single agent for lymphoid malignancies including CLL.[14-17] On the basis of results of a randomized phase III trial comparing chlorambucil alone with bendamustine alone in first-line treatment of CLL, bendamustine was recently approved for CLL in the United States and in several European countries.[18] In a small phase I/II trial including 16 patients with relapsed or refractory CLL, nine (56%) of 16 patients responded to single-agent bendamustine, whereas the dose had to be de-escalated as a result of severe toxicity in three patients.[19] In vitro studies in primary CLL cells have demonstrated synergistic proapoptotic effects of bendamustine plus the anti-CD20 antibody rituximab (BR).[20] Encouraging clinical results have been obtained using BR combination treatment in relapsed, refractory, and previously untreated non-Hodgkin's lymphoma.[21,22] Patients across different lymphoma subtypes responded, with an overall response rate (ORR) of 90% to BR therapy. In light of these promising preclinical and clinical data, we initiated this phase II trial to evaluate the safety and efficacy of BR combination treatment in patients with relapsed and/or refractory CLL.

PATIENTS AND METHODS

Study Design and Objective

This prospective, multicenter, nonrandomized, phase II study was approved by the competent institutional review board and conducted in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines (ClinicalTrials.gov identifier: NCT00274989). All patients provided written informed consent. The primary end point is ORR. Secondary end points include toxicity, quality and duration of response, event-free survival (EFS), minimal residual disease (MRD) levels, and ORR in biologically defined risk groups.

Patients and Treatment Schedule

Patients included in the trial had to be diagnosed with CLL in need of treatment according to the National Cancer Institute (NCI) guidelines and had to have relapsed and/or refractory disease (refractory was defined as no complete or partial remission after therapy or as progression within 6 months).[23] Eligible patients had received at least one but not more than three previous treatments, were at least 18 years of age, and had a WHO performance status of 0 to 2, a life expectancy of at least 12 weeks, and adequate renal (creatinine clearance $>$ 30 mL/min) and liver function (total bilirubin and transaminases $\leq 2\times$ the institutional upper limit of normal value).

All patients were scheduled to receive bendamustine 70 mg/m^2 on days 1 and 2 combined with rituximab 375 mg/m^2 on day 0 for the first course and 500 mg/m^2 on day 1 for all subsequent courses based on previously published data for fludarabine, cyclophosphamide, and rituximab.[7,19] Treatment was administered every 28 days for up to six courses depending on response and toxicity.

Assessments

Patients underwent baseline assessment before first treatment. During therapy, assessment for adverse events and myelosuppression was performed weekly. After three courses of treatment, an interim response assessment was performed. Patients who had achieved at least stable disease with acceptable toxicity continued to receive study treatment for three additional courses. Restaging after completion of therapy was performed 1 month $\pm$ 7 days after the start of the last course of therapy and had to be reconfirmed 2 months later. Subsequently, patients completed follow-up examinations every 3 months for the ensuing 36 months.

Criteria for Response and Toxicity

Response was determined according to the NCI Working Group 1996 criteria for CLL, including bone marrow examination and radiographic confirmation of complete response (CR).[23] Responses and disease progression were assessed by the study investigators and verified by a central, investigator-independent medical review. The response achieved after termination of therapy had to be maintained for at least 2 months. Radiographic imaging was performed at the discretion of the treating physician at screening and during interim staging and follow-up. Treatment toxicity was reported by the investigators according to NCI Common Terminology Criteria for Adverse Events version 3.0.[24]

Biologic Prognostic Markers

Analysis of genomic aberrations by interphase fluorescence in situ hybridization, *IGHV* mutational status by sequencing, MRD by four-color flow cytometry of peripheral blood and bone marrow, and expression of CD38 and ZAP-70 by fluorescence-activated cell sorting were performed in the central reference laboratories of the German CLL Study Group.[25,26]

Statistical Methods

The sample size estimation was performed according to the Simon two-stage optimal design with type I error of $\alpha = .05$, to conclude the efficacy of an uninteresting regimen (response rate $<$ 50%), and a type II error of $\beta = .10$, implying the rejection of an active regimen (response rate $>$ 70%).[27] The primary end point of ORR was calculated in the intent-to-treat (ITT) population, which was defined as all patients who received at least one dose of study medication. Secondary end points were the ORR in biologically defined risk groups and MRD response rate, as well as the duration of response defined as the time period between the first documentation of response and the initial documentation of progressive disease or death as a result of any cause. An additional secondary end point was EFS, which was defined as the date of first treatment with BR to the date of progressive disease, the beginning of new treatment for any hematologic malignancy, or death as a result of any cause. Median EFS and duration of response in the ITT population were estimated using the Kaplan-Meier method.

RESULTS

Patient Characteristics

Between March 2006 and June 2007, 83 patients were registered for trial participation at 32 centers in Germany. Five patients had to be excluded from the trial as a result of missing informed consent (n = 3) or diagnosis other than CLL (small lymphocytic lymphoma, n = 1; immune thrombocytopenic purpura, n = 1). Seventy-eight patients with a median age of 66.5 years (range, 42 to 86 years) received at least one dose of treatment and constitute the ITT population for basic characteristics, safety, and efficacy analysis (Table 1). Twenty-nine patients (37.2%) were 70 years of age or older, and 48% of patients presented with Binet stage C at study entry. The median number of previous therapies applied was two.

Sixty-three patients (80.8%) had previously received fludarabine alone and/or fludarabine-containing combination therapies, seven patients (9.0%) had received rituximab-containing therapies, and five patients (6.4%) had received alemtuzumab-containing regimens. Two patients had undergone autologous stem-cell transplantation before trial participation. Twenty-two patients (28.2%) were refractory to fludarabine (Table 2).

The patient population had a high incidence of unfavorable genetic markers; a deletion of chromosome 17p13 [del(17p)] was detected in 17.9% of the patients, del(11q) was detected in 20.5% of patients, and unmutated *IGHV* was detected in 65.4% of the patients. Moreover, 42.3% of the patients were characterized by high levels of serum thymidine kinase ($>$ 10 U/L; median, 20.0 U/L; range, 5.0 to 519.0 U/L), and 47.4% of patients presented with high levels of serum β_2-microglobulin ($>$ 3.5 mg/L; median, 4.3 mg/L; range, 0.7 to 8.5 mg/L). Thirty-three patients (42.3%) had a creatinine clearance $\leq$ 70 mL/min (Table 1).

Table 1. Patient Demographic and Baseline Clinical Characteristics

Characteristic	No. of Patients (N = 78)	%
Sex		
Male	51	65.4
Female	27	34.6
Age, years		
Median	66.5	
Range	42-86	
< 65	24	30.8
65-69	25	32.1
≥ 70	29	37.2
Previous No. of therapies for CLL		
Median	2	
Range	1-5*	
1	36	46.2
2	22	28.2
3	18	23.1
WHO performance status (n = 75)		
0	33	42.3
1	40	51.3
2	2	2.6
Creatinine clearance, mL/min		
Median	75.2	
Range	31.8-159.2	
≤ 70	33	42.3
> 70	45	57.7
Binet stage (n = 75)		
A	14	18.7
B	25	32.1
C	36	48.0
Presence of B symptoms (n = 77)		
Yes	27	35.1
No	50	64.9
WBC count, $\times 10^3/\mu$L (n = 77)		
Median	45.3	
Range	1.8-597.0	
≤ 50	42	53.8
> 50	35	44.9
Absolute lymphocyte count, $\times 10^3/\mu$L		
Median	40.4	
Range	1.6-585.1	
Hemoglobin, g/dL (n = 77)		
Median	12.1	
Range	5.2-16.2	
≤ 10.0	14	18.2
> 10.0	63	81.8
Platelets, $\times 10^3/\mu$L (n = 77)		
Median	109.0	
Range	12.0-361.0	
< 100.0	30	39.0
≥ 100.0	47	61.0
Serum thymidine kinase, U/L (n = 41)		
Median	20.0	
Range	5.0-519.0	
≤ 10.0	8	19.5
> 10.0	33	80.5
Serum β_2-microglobulin, mg/L (n = 55)		
Median	4.3	
Range	0.7-8.5	
≤ 3.5	18	32.7
> 3.5	37	63.3
Expression of ZAP-70, % (n = 49)		
Median	8.0	
Range	0.0-90.0	
≤ 20	37	75.5
> 20	12	24.4
Expression of CD38, % (n = 48)		
Median	28.1	
Range	0.2-92.1	
≤ 30	24	50.0
> 30	24	50.0
Genomic aberrations by FISH (n = 73)		
17p deletion	14	17.9
11q deletion†	16	20.5
13q deletion‡	21	26.9
Trisomy 12§	6	7.7
Normal‖	16	20.5
IGHV mutational status (n = 76)		
Mutated	25	32.9
Unmutated	51	67.1

Abbreviations: CLL, chronic lymphocytic leukemia; FISH, fluorescent in situ hybridization.
*There were two protocol violators; one patient had four previous therapies, and one patient had five previous therapies for CLL.
†Not including 17p deletion.
‡Not including 17p deletion or 11q deletion.
§Not including 17p deletion, 11q deletion, or 13q deletion.
‖Not including 17p deletion, 11q deletion, 13q deletion, or trisomy 12 (ie, genetic classification according to hierarchical model).

Treatment

A total of 353 treatment courses were administered (median number per patient, six courses); 44 patients (56.4%) received the full six courses of therapy, and 60 patients (76.9%) received at least three courses. In total, 49 patients (62.8%) received prophylactic antibiotics. Granulocyte colony-stimulating factor was administered in 10 patients (12.8%) for a median duration of 4.5 days. Dose reductions of any of the two drugs by more than 10% of the planned dose were applied in 29 patients (37.2%) mostly as a result of treatment-related hematologic toxicity, particularly neutropenia. Nineteen patients (24.4%) had a dose reduction of rituximab alone, 18 patients (23.1%) had a reduction of bendamustine alone, and five patients (6.4%) had dose reductions of both rituximab and bendamustine. Treatment was discontinued early in 34 patients (43.6%) as a result of withdrawal of consent (n = 9), toxicity (n = 15), progressive disease (n = 8), and other reasons (n = 2).

Safety

After a median follow-up time of 24 months, the following 28 deaths occurred: 21 patients died unrelated to treatment in disease progression, including six patients who died during or after subsequent treatment of CLL and four patients were diagnosed with Richter's transformation after the end of treatment. Three other deaths were also unrelated to treatment, including one caused by pre-existing histiocytic sarcoma, one caused by cardiac insufficiency, and one for which the cause was unknown. Four patients died during study treatment; one patient died as a result of osteomyelitis unrelated to treatment, and three patients (3.8%; including one patient with pre-existing Richter's transformation) died

Table 2. Previous Therapies for CLL

Previous Therapy	No. of Patients	Refractory Patients	
		No.	%
No. of patients with at least one previous treatment containing fludarabine	63	21	33.3
Fludarabine monotherapy	34	13	38.2
Fludarabine and cyclophosphamide	32	8	25.0
Fludarabine, cyclophosphamide, and mitoxantrone	4	1	25.0
Fludarabine, cyclophosphamide, and rituximab	3	1	33.4
Fludarabine and alemtuzumab	3	1	33.4
Fludarabine and rituximab	2	0	0
Fludarabine, cyclophosphamide, and alemtuzumab	1	0	0
Fludarabine and mitoxantrone	1	1	100.0
CHOP-like	7		
CHOP-like plus rituximab	1		
Chlorambucil monotherapy or with corticosteroids	32		
Bendamustine monotherapy	3		
Cyclophosphamide monotherapy or with corticosteroids	2		
Rituximab monotherapy	1		
Alemtuzumab monotherapy	1		
Autologous peripheral-blood stem-cell transplantation	2	0	0
Radiotherapy	2	1	50.0

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CLL, chronic lymphocytic leukemia.

Table 3. Incidence of CTCAE Grade 3 or 4 Adverse Events

Adverse Event	Grade 3		Grade 4	
	No.	%	No.	%
Adverse events according to treatment courses (n = 353)				
Total courses with at least one grade 3 or 4 event	52	14.7	40	11.3
Hematologic toxicity	46	13.0	40	11.3
Leukopenia	17	4.8	6	1.7
Neutropenia	29	5.4	17	4.8
Thrombocytopenia	23	6.5	19	5.4
Anemia	13	3.7	13	3.7
Tumor lysis syndrome	0	0	0	0
Hemolysis	2	0.6	0	0
Allergic reaction	2	0.6	0	0
Infections	12	3.4	0	0
Other nonhematologic toxicities	14	4.0	3	0.8
Adverse events according to patients (n = 78)				
Total patients with at least one grade 3 or 4 event	21	26.9	19	24.4
Hematologic toxicity	19	24.4	20	25.6
Leukopenia	8	10.3	6	7.7
Neutropenia	7	9.0	11	14.1
Thrombocytopenia	11	14.1	11	14.1
Anemia	9	11.5	4	5.1
Tumor lysis syndrome	0	0	0	0
Hemolysis	2	2.6	0	0
Allergic reaction	2	2.6	0	0
Infections	10	12.8	0	0
Other nonhematologic toxicities	9	11.5	2	2.6

Abbreviation: CTCAE, Common Terminology Criteria for Adverse Events, version 3.0.

during the first two courses of treatment from infections that were related to therapy (septicemia, n = 2; pneumonia, n = 1).

In the ITT population, 46 patients (59.0%) experienced at least one grade 3 or greater adverse event during treatment or up to 2 months thereafter. The most common adverse events were hematologic toxicities, occurring in 39 patients (50.0%); severe neutropenia, thrombocytopenia, and anemia were observed in 18 patients (23.1%), 22 patients (28.2%), and 13 patients (16.6%), respectively (Table 3). According to treatment courses, severe neutropenia, thrombocytopenia, and anemia were observed in 10.2%, 11.9%, and 7.4% of all applied treatment courses, respectively (Table 3).

Two patients entered the study with an active hemolysis, and both patients were stabilized under treatment with BR. Infection was the most common nonhematologic toxicity. Grade 3 severe infections, mainly febrile neutropenia and pneumonia, occurred in 10 patients (12.8%), but no grade 4 infections were seen. Other nonhematologic toxicities are listed in Table 3.

Of note, the incidence of adverse events, particularly leukopenia, was significantly higher in patients with a creatinine clearance $\leq$ 70 mL/min compared with patients with normal renal function (P = .031 and P = .019, respectively). Patients with a lower level of creatinine clearance, compared with patients with a creatinine clearance greater than 70 mL/min, required more dose reductions (42.4% *v* 26.7%, respectively; P = .35) and experienced more infections (27.3% *v* 13.3%, respectively; P = .15).

Treatment Efficacy

In the ITT population, the ORR was 59.0% (95% CI, 47.3% to 70.0%; n = 46), with a CR rate of 9.0% (n = 7), two nodular partial responses, and a partial response rate of 47.4% (n = 37). Stable disease was observed in 20 patients (25.6%), and five patients (6.4%) had progressive disease. In seven patients (9.0%), no response assessment was performed because of early death before interim staging (n = 4), withdrawal of consent after first course of therapy (n = 2), or loss to follow-up after the second course (n = 1). Excluding these patients from analysis, the ORR and CR rates were 64.8% and 9.9%, respectively.

After a median follow-up time of 24.0 months, the median EFS time was 14.7 months (95% CI, 14.1 to 20.1 months). Median EFS was reached at 13.8 months for patients with Binet stage C, at 20.5 months for patients with Binet stage B, and at 27.5 months for patients with Binet stage A. The median progression-free survival time was 15.2 months (95% CI, 12.5 to 17.9 months), and the median overall survival time was 33.9 months (95% CI, 25.5 to 42.1 months). Among the 46 responders, the median duration of response was 15.2 months (95% CI, 12.1 to 18.3 months; Fig 1).

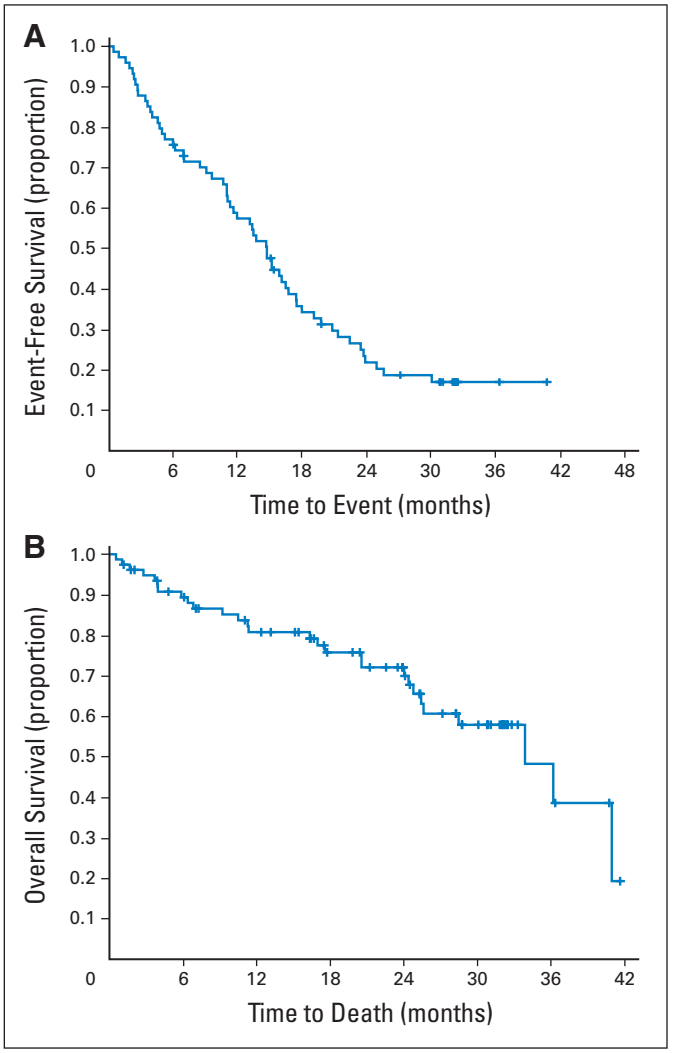

Fig 1. (A) Event-free survival and (B) overall survival for all patients (intent-to-treat population).

Table 4. Survival Analysis

Factor	No. of Patients	OS Median (months)	OS *P*	PFS Median (months)	PFS *P*	EFS Median (months)	EFS *P*
All patients	78	33.9	—	15.197	—	14.7	—
Genetic subgroup							
17p deletion	14	16.3	.007	6.8	.19	4.8	.044
11q deletion*	15	NR		15.9		15.9	
Trisomy 12†	5	20.5		16.9		10.7	
13q deletion‡	19	41.0		17.5		17.5	
No abnormalities according to the hierarchical model§	16	33.9		16.7		13.8	
IGVH status							
Unmutated	49	25.6	.009	13.8	.025	13.2	.013
Mutated	23	NR		17.5		17.5	
Binet stage							
A	13	NR	.93	17.5	.7	15.9	.831
B	24	33.9		14.7		14.7	
C	34	NR		15.2		13.8	
Age, years							
≤ 70	46	33.9	.9	14.7	.9	13.7	.952
> 70	28	NR		17.0		15.1	
No. of previous therapies							
≤ 2	56	36.2	.02	16.5	.07	15.2	.198
> 2	18	24.0		11.6		11.6	
Serum β_2-microglobulin, mg/L							
≤ 3.5	17	41.0	.1	13.2	.9	13.2	.678
> 3.5	36	25.6		14.7		12.0	
Serum thymidine kinase, U/L							
≤ 10.0	8	25.4	.8	13.2	.7	11.0	.605
> 10.0	32	33.9		12.0		11.6	

Abbreviations: EFS, event-free survival; NR, not reached at time of analysis; OS, overall survival; PFS, progression-free survival.
*Not including 17p deletion.
†Not including 17p deletion or 11q deletion.
‡Not including 17p deletion, 11q deletion, or trisomy 12.
§Not including 17p deletion, 11q deletion, 13q deletion, or trisomy 12 (ie, genetic classification according to hierarchical model).

Treatment Efficacy in Prognostic Subgroups

Pretreatment characteristics and clinical and biologic parameters were evaluated for correlations with ORR. ORR was significantly associated with higher cumulative doses of bendamustine and rituximab (bendamustine, $P < .001$; rituximab, $P = .001$), lower level of serum thymidine kinase (≤ 10.0 U/L; $P = .044$), lower level of β_2-microglobulin (≤ 3.5 mg/L; $P = .035$), and lower number of previous therapies ($P = .046$). In a Cox regression analysis, the number of previous therapies was found to be a predictor of EFS (hazard ratio, 1.4; 95% CI, 1.023 to 1.790; $P = .034$). In terms of the prognostic factors CD38 and ZAP-70, a significance level was not achieved using the established cutoff levels for CD38 and ZAP-70.

Significant differences in response to treatment were observed among the genetic subgroups ($P = .006$); 12 (92.3%) of 13 patients with del(11q) achieved a remission, with one patient (7.7%) achieving a CR. All four patients with trisomy 12 responded with a partial response, whereas in the high-risk group with del(17p), one (7.1%) of 14 patients responded with a CR and had a response duration of 17.0 months. Kaplan-Meier analysis showed significant differences for EFS, as listed in Table 4 and shown in Figure 2 ($P = .044$). Twenty-seven (58.6%) of 46 patients with unmutated *IGHV* status were responsive to treatment, with a CR rate of 4.3%. For EFS, Kaplan-Meier analysis indicated significant differences ($P = .013$). In Cox regression analysis, the presence of *IGHV* unmutated status was a negative prognostic indicator of EFS (hazard ratio, 2.2; 95% CI, 1.157 to 4.031;

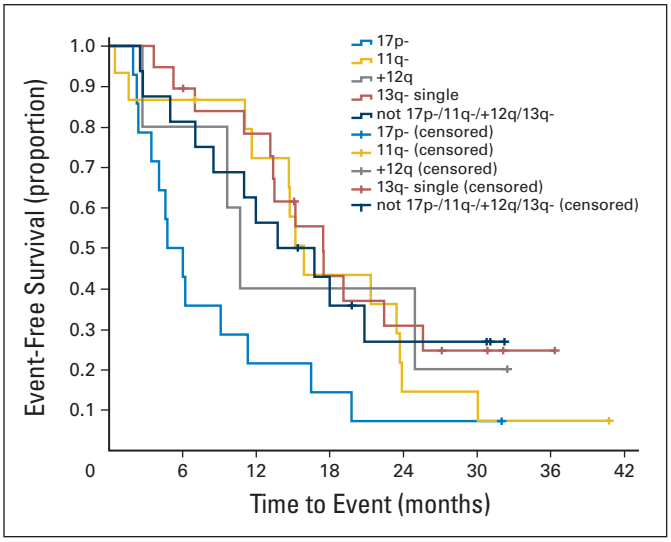

Fig 2. Event-free survival in cytogenetic subgroups.

Table 5. Response to Treatment

Population	No. of Patients	Missing*		CR		PR/nPR		SD		PD		OR		
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	*P*
Total patients	78	7	9.0	7	9.0	39	50.0	20	25.6	5	6.4	46	59.0	—
Genetic subgroup														
Patients with cytogenetic results and response assessment	67			6	9.0	36	53.7	20	29.9	5	7.5	42	62.7	—
17p deletion	14			1	7.1	0	0	10	71.4	3	21.4	1	7.1	.006
11q deletion†	13			1	7.7	11	84.6	1	7.7	0	0	12	92.3	
Trisomy 12‡	4			0	0	4	100.0	0	0	0	0	4	100.0	
13q deletion§	20			2	10.0	13	65.0	5	25.0	0	0	15	75.0	
No abnormalities according to the hierarchical model‖	16			2	12.5	8	50.0	4	25.0	2	12.5	10	62.5	
IGHV status														
Unmutated	46			2	4.3	25	54.4	15	32.6	4	8.7	27	58.7	.053
Mutated	23			4	17.4	14	60.9	5	21.7	0	0	18	78.2	
Fludarabine subgroup														
Patients naive to fludarabine	12			4	33.3	8	66.7	0	0	0	0	12	100.0	.01
Patients sensitive to fludarabine	38			3	7.9	20	52.7	10	26.3	3	7.9	23	60.5	
Patients refractory to fludarabine	22	1	4.5	0	0	10	45.5	9	40.9	2	9.1	10	45.5	
Previous therapy with rituximab and/or alemtuzumab	10			0	0	6	60.0	4	40.0	0	0	6	60.0	.73
Prognostic subgroup														
Binet stage														
A	12			2	16.7	7	58.3	3	25.0	0	0	9	75.0	.56
B	24			1	4.2	13	54.2	7	29.2	3	12.5	14	58.3	
C	32			4	12.5	18	56.3	8	25.0	2	6.3	22	68.8	
Age, years														
≤ 70	45			4	8.9	23	51.1	15	33.3	3	6.7	27	60.0	.76
> 70	26			3	11.5	16	61.5	5	19.2	2	7.7	19	73.1	
No. of previous therapies														
≤ 2	53			7	13.2	30	56.6	11	20.8	5	9.4	37	69.8	.08
> 2	18			0	0	9	50.0	9	50.0	0	0	9	50.0	
Serum β_2-microglobulin, mg/L														
≤ 3.5	16			2	12.5	11	68.8	2	12.5	1	6.3	13	81.3	.035
> 3.5	35			3	8.6	14	40.0	15	42.9	4	7.8	17	48.6	
Serum thymidine kinase, U/L														
≤ 10.0	8			0	0	7	87.5	1	12.5	0	0	7	87.5	.044
> 10.0	31			3	9.7	10	32.3	13	41.9	5	16.1	13	41.9	

Abbreviations: CR, complete response; nPR, nodular partial response; OR, overall response; PD, progressive disease; PR, partial response; SD, stable disease.
*No. of patients without response assessment.
†Not including 17p deletion.
‡Not including 17p deletion or 11q deletion.
§Not including 17p deletion, 11q deletion, or trisomy 12.
‖Not including 17p deletion, 11q deletion, 13q deletion, or trisomy 12 (ie, genetic classification according to hierarchical model).

$P = .016$). MRD levels in peripheral blood of 27 evaluable patients showed that two patients (7.4%) had MRD levels less than 10^{-4}, whereas one (7.7%) of 13 evaluable patients achieved MRD negativity in bone marrow.

Fludarabine-naive and fludarabine-sensitive patients had a better ORR with a longer median response duration than fludarabine-refractory patients ($P = .01$). Of the 22 patients (28.2%) with fludarabine-refractory disease, 10 patients (45.5%) responded, with a median response duration of 8.7 months (95% CI, 8.2 to 9.1 months). Fludarabine-sensitive patients had an ORR of 60.5% with a median response duration of 15.2 months (95% CI, 11.2 to 19.2 months), whereas the ORR of fludarabine-naive patients was 100%, with a median response duration of 16.8 months (95% CI, 10.8 to 22.8 months). Six (60%) of 10 patients who had received a previous treatment containing antibody showed a partial response, as described in Table 5. Of the seven patients who had previously received rituximab, five patients (71.4%) responded with a partial remission.

DISCUSSION

The addition of rituximab to chemotherapeutic regimens has significantly improved the treatment impact for both previously untreated patients and patients with relapsed CLL.[7-9,11] This phase II trial is the first trial, to our knowledge, prospectively assessing safety and efficacy of bendamustine in combination with rituximab in a high-risk population of patients with relapsed and/or refractory CLL.

The study population included a significant proportion of patients (accounting for > 25% of the entire study population) with fludarabine-refractory disease as well as a high rate of patients with

del(17p) (17.9%) and with unmutated *IGHV* status (65.4%). Moreover, 48.0% of the study population presented with Binet stage C, and almost half of the patients (42.3%) had an impaired renal function.

In light of these high-risk characteristics of the patient population included in our trial, the reported ORR of 59.0%, including a 9.0% CR rate, compares favorably with the results achieved using monotherapy with bendamustine as well as treatment with alemtuzumab in patients with refractory CLL disease.[13,19] Of note, the results are based on a strict ITT analysis including patients without response assessment. Treatment efficacy was found to be dependent on the cumulative dose of study treatment. According to the re-treatment criteria described previously, only 56.4% of the patients received the planned six treatment courses. The main reason for treatment discontinuation was toxicity, mostly hematologic toxicity.

Compared with a recently published trial evaluating the efficacy of fludarabine, cyclophosphamide, and rituximab (FCR) and reporting an ORR based on investigator assessment of 69.9% with a CR rate of 24.3% in relapsed CLL, the response data derived from our trial seem inferior; however, it is important to emphasize that the aforementioned protocol included fludarabine-sensitive patients after first-line treatment only.[10] Interestingly, the authors reported an ORR of 61% with a CR rate of 9% when analysis was performed on response data assessed by an independent review committee.[10] Because we also performed an investigator-independent medical review, these results compare favorably with the response rates derived in our trial.

Compared with trials using FCR in relapsed CLL, the observed adverse effects of the BR regimen compare favorably, even though the study population in our trial displayed poorer risk factors. Whereas in our study only 23.1% of patients treated with BR experienced severe neutropenia of grade 3 or greater, this rate was reported to be between 42% per patient and 62% per course in the trials evaluating FCR. The reported low rate of severe infections of 12.8% in patients receiving BR treatment also compares favorably with FCR. Importantly, other nonhematologic adverse effects were rare. In particular, no neurologic adverse effects and allergic skin reactions were observed in our trial, although they have occasionally been reported in other trials using bendamustine treatment.[28,29]

For fludarabine-refractory patients, the ORR of 45.5% achieved in our trial is worth noting. In comparison, the monoclonal anti-CD20 antibody rituximab, when used in addition to fludarabine and cyclophosphamide, achieved a CR rate and ORR of 6% and 58%, respectively, in the same high-risk patient population.[9]

In conclusion, we describe a representative population of patients with CLL, including many elderly and high-risk patients with advanced disease, receiving treatment with BR for their relapsed disease. Except for patients with del(17p) who did not benefit from the treatment regimen, the combination therapy of BR offers an effective and safe treatment for patients with relapsed CLL. Further studies with well-defined patient populations need to be performed to validate these findings. Given the promising results obtained in this phase II trial, we initiated a further phase II trial evaluating the BR regimen as first-line therapy for patients with CLL.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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