Routine Use of Bendamustine in Patients with Chronic Lymphocytic Leukemia: An Observational Study

MARIJANA NINKOVIC[1], MICHAEL FIEGL[1], MICHAEL MIAN[2], PATRIZIA MONDELLO[3], FLORIAN KOCHER[1], CHRISTIAN WALDTHALER[1], IRMGARD VERDORFER[4], MICHAEL STEURER[1], GÜNTHER GASTL[1] and ANDREAS PIRCHER[1]

[1]*Department of Internal Medicine V, Hematology and Oncology, Medical University of Innsbruck, Innsbruck, Austria;*
[2]*Department of Hematology and CBMT, Hospital of Bolzano, Bolzano, Italy;*
[3]*Department of Human Pathology, University of Messina, Messina, Italy;*
[4]*Department of Medical Genetics, Molecular and Clinical Pharmacology, Medical University of Innsbruck, Innsbruck, Austria*

Abstract. *Bendamustine is an established treatment option in chronic lymphocytic leukemia (CLL) and frequently used in Austria and Italy. Therefore, we analyzed 100 unselected, consecutive patients with CLL (treatment-naïve and relapsed/refractory) receiving bendamustine in a real-life setting. Most patients were treated with bendamustine in combination with rituximab (BR). However, bendamustine monotherapy was additionally evaluated. Patients treated with BR had a significantly higher overall response rate of 76% (complete response=22%) when compared to those treated solely with bendamustine (overall response rate=50%; complete response=13%). Overall survival (OS) and progression -ree survival (PFS) were significantly lower in the bendamustine-treated group (OS=14.3 months; PFS=8.3 months) compared to the BR group (OS=42.7; PFS=22.5 months; both p<0.001). In multivariate analysis, patients with a good cytogenetic risk and those receiving BR had a significantly better OS. Grade 3/4 hematological complications were seen in 32% of the patients. Hence, bendamustine, especially in combination with rituximab, is an effective therapy with manageable toxicity for non-selected patients with CLL including those pre-treated with fludarabine and the elderly.*

Correspondence to: Marijana Ninkovic, MD, Department of Internal Medicine V/Hematology and Oncology, Medical University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria. Tel: +43 512-50480761, Fax: +43 51250422602, e-mail: marijana.ninkovic@tirol-kliniken.at

Key Words: Bendamustine, rituximab, chronic lymphocytic leukemia (CLL), chemotherapy, immunotherapy

Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia in industrialized countries (1, 2). Despite the advent of new drugs (3, 4), it still remains an incurable disease, except for the few patients who are able to undergo allogeneic stem-cell transplantation (5-8). Only recently, bendamustine, first synthesized in the early 1960s, was to become an efficient treatment for hematological malignancies and it was approved for rituximab-refractory indolent lymphoma (9), CLL (10) and multiple myeloma (11).

Up until now, the use of bendamustine in CLL was mainly investigated in clinical trials in combination with rituximab (12). Current guidelines recommend a bendamustine-based therapy in patients with Binet stage C or Binet stage A and B with symptomatic disease and notably physically non-fit patients (11, 13, 14). Only a few real-life CLL populations, which were either treated with bendamustine monotherapy or in combination with rituximab, have been analyzed retrospectively (2, 13). Therefore, we herein present data on the toxicity and therapeutic efficacy in 100 unselected, consecutive patients with treatment-naïve or relapsed/refractory CLL treated with bendamustine-based therapy.

The objectives of the present study were to provide a descriptive analysis of a CLL population who received bendamustine-based therapy to evaluate patients' characteristics, including the spectrum of bendamustine-based therapy combinations used, and efficacy in terms of response and survival; as well as to determine toxicity in routine clinical use.

Patients and Methods

We retrospectively assessed 100 unselected, consecutive patients with CLL who received bendamustine either as monotherapy (n=24) or in combination with rituximab (n=76). The study was conducted according to the rules of the Medical University of Innsbruck Ethics Committee, as reported in other studies (15-17).

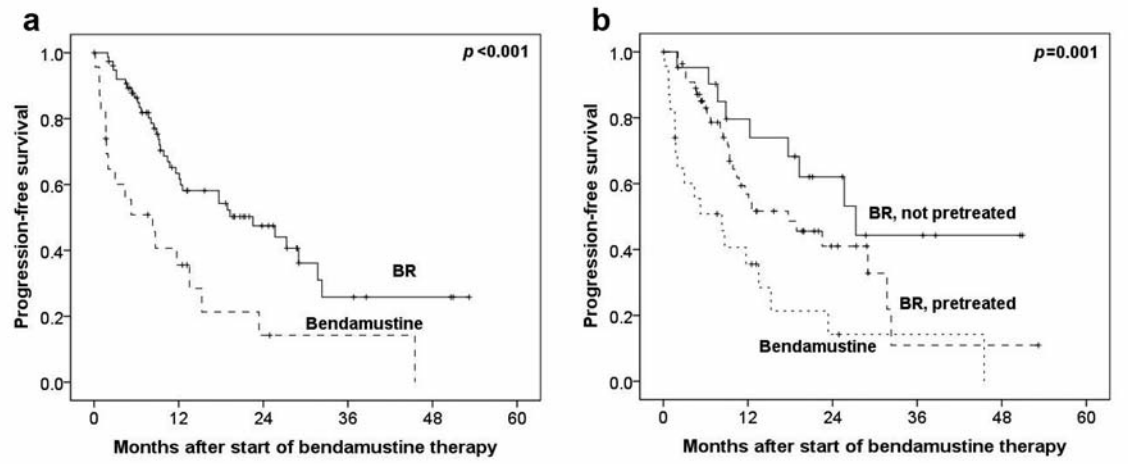

Figure 1. *Progression-free survival according to treatment modality. a: The median PFS for patients treated with bendamustine and rituximab (BR) was 22.5 months (n=76) compared to 8.3 months for patients treated with bendamustine monotherapy (n=24; p<0.001). b: The median PFS for treatment-naïve patients on BR was 27.3 months (n=21) and for pre-treated patients was 17.7 months (n=55), while that of patients on bendamustine monotherapy was 8.3 months (n=24). When comparing all groups together the p-value was 0.001 (bendamustine vs. BR pre-treated [p=0.010], bendamustine vs. BR not pre-treated [p=0.001], BR pre-treated vs. BR not pre-treated [p=0.120]).*

Data concerning the therapeutic regimen, efficacy, toxicity and follow-up were obtained directly from the clinical charts and from primary physicians. Data for 26 patients from a previous evaluation were updated and included in the study (18). The final data update was in September 2013. All analyses were performed using SPSS software version 20 (IBM Corp. Armonk, NY, USA).

Complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD) were classified according to the current International Work Group on Chronic Lymphocytic Leukemia guidelines for CLL (iWCLL) (7). Treatment-related adverse events were classified according to the Common Toxicity Criteria (CTC v.4) (19). Survival curves were plotted according to the Kaplan–Meier method. Progression-free survival (PFS) was defined as the time from the initiation of bendamustine-based therapy until disease progression (PD) or death, whichever occurred first. Patients, who were lost to follow-up without any sign of progression, or were alive at the final data update, were censored at the time of last observation. Overall survival (OS) was measured from start of bendamustine-based therapy. Univariate survival comparisons between categorical variables were evaluated with the log-rank test. All parameters with a *p*-value of 0.05 or less in univariate analyses were included in a Cox proportional hazard model. The *p*-value of 0.05 or less was considered as significant in two-sided tests.

A subset of 41 patients' assessments of co-morbidity according to the cumulative illness rating scale (CIRS) was available. The patient fitness groups according to CIRS were defined by the Alberta Health Services clinical practice guideline LYHE-007 (20).

Results

Patients' characteristics and mode of therapy. Forty-five patients (45%) were treated in Tyrol (Austria), and 31 (31%) and 24 (24%) in Bolzano and Messina (Italy), respectively. Bendamustine-based therapy was initiated between August 2007 and April 2013. Detailed patient characteristics are shown in Table I. The administered bendamustine dose ranged between 60 mg/m^2 and 120 mg/m^2 body surface area on day 1 and 2 every 4 weeks. Rituximab was usually administered on day one of each cycle, mostly at the recommended dose of 500 mg/m^2. The median number of cycles administered was 5 in the BR group and 3.5 cycles in the bendamustine-treated group.

Bendamustine was administered as monotherapy (n=24, 24%) or in combination with rituximab (n=76, 76%). A total of 64 patients were male (64%). The median age was 73 years (range=41-88 years). The majority of the cases were treated in a higher therapy line (76%) and presented with Rai stages III and IV (27% and 40%, respectively), and were characterized by CIRS scores over 6 [n=32/41 evaluable patients (78%), median CIRS = 9].

Cytogenetic status, by fluorescence *in situ* hybridization (FISH) was available in 81 patients. According to the algorithm of Döhner *et al*. (21), 44% of patients were high-risk, harboring 11q deletion (n=15) or 17p deletion (n=20), meaning that patients with these cytogenetic aberrations usually respond very poorly to conventional chemotherapy and have inferior survival. Most of the patients had a WHO performance status of 0-1 (87%) and presented without B-symptoms (61%) at the beginning of the treatment. Forty-three patients with CLL (43%) had previously received fludarabine, of whom seven (16%) had fludarabine-refractory disease according to the criteria proposed by Keating *et al*. (22). Bendamustine alone or in association with rituximab was administered in first, second and higher line of therapy in 24 (24%), 27 (27%) and 49 (49%) patients, with a median of one (range=0-7) previous therapy.

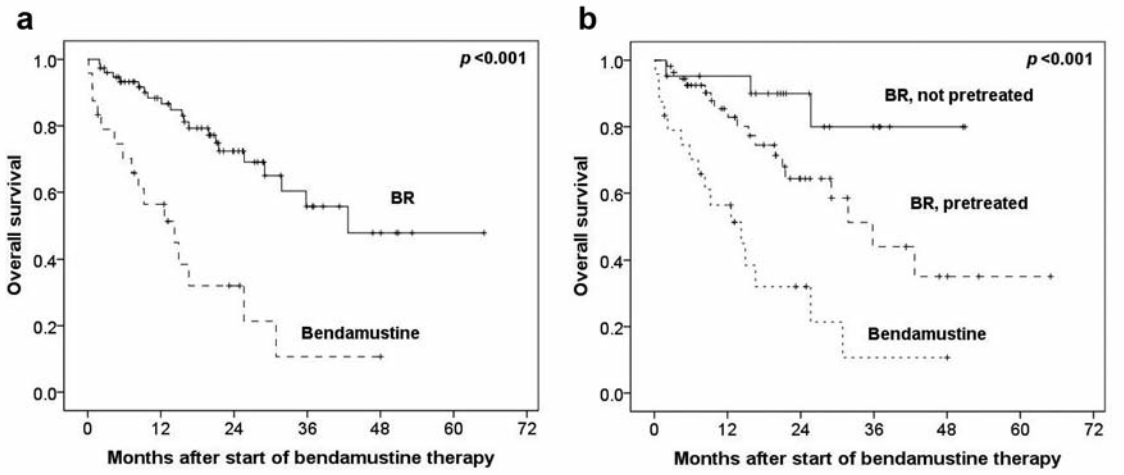

Figure 2. *Overall survival (OS) according to treatment modality. a: The median OS in patients treated with bendamustine and rituximab (BR) was 42.7 months (n=76), compared to 14.3 months for those treated with bendamustine monotherapy (n=24; p<0.001). b: The median OS for treatment-naïve patients on BR was not reached (n=21) and for pre-treated patients on BR was 35.8 months (n=55), while that for patients on bendamustine monotherapy was 14.3 months (n=24). When comparing all groups together the p-value was lower than 0.001 (bendamustine vs. BR pre-treated [p=0.001], bendamustine vs. BR not pre-treated [p<0.001], BR pre-treated vs. BR not pre-treated [p=0.050]).*

Response to therapy. Sufficient data for response assessment information were available for 87 patients. In 13 patients, response was not evaluable due to premature death (n=6), therapy switch (n=1), premature discontinuation due to toxicity (n=2), or due to loss to follow-up (n=4) (as shown in Table II). A response according to iWCLL criteria was achieved in 70 patients (ORR=70%) with CR in 20 (20%) and PR in 50 (50%). SD and PD, under therapy or within 2 months after the last dose of bendamustine, was observed in six (6%) and 11 (11%) cases, respectively. For response analysis, we divided the cohort into two subgroups, the group who received bendamustine in combination with rituximab and those who received solely bendamustine. Interestingly, patients receiving BR were characterized by significantly higher response, with ORR achieved in 58 patients (76%) (CR in 17 [22%]) than patients receiving solely bendamustine, with ORR in 12 (50%) patients, (CR in 3 [13%]) (p=0.014).

In the BR group, the most important factors determining ORR were cytogenetic risk category (p=0.004), the Rai staging (p=0.042) and the cumulative dose of bendamustine (p=0.005). Furthermore, patients over the age of 75 years, as well as the ones with a CIRS score greater than 6 and those with GFR less than 60 ml/min did not have any disadvantages in ORR compared to younger patients, those with a lower CIRS score and those with higher GFR. The bendamustine-treated cohort showed similar results, but some significant results concerning β2-microglobulin levels and CIRS score have to be regarded carefully, due to their one-sided distribution (shown in Table III).

Progression-free survival. The overall median PFS was 17.7 months. For the whole cohort, PFS was influenced by clinical characteristics and therapy-related factors, namely the FISH karyotype risk-group (p<0.001), type of bendamustine therapy (p<0.001), quality of response (p<0.001) and cumulative dose of applied bendamustine (p=0.005). The cohort treated with BR regimen reached a median PFS of 22.5 months, which is significantly longer than the median PFS of the cohort treated with bendamustine monotherapy (8.3 months; p<0.001; Figure 1a). When analyzing therapy line-related sub-groups, treatment-naïve patients (n=21) in the BR cohort had a tendency for a longer median PFS (27.3 months) than pre-treated patients in this cohort (n=55; PFS=17.7 months; p=0.120). Due to a relatively small cohort treated with first-line bendamustine monotherapy (n=3), this subgroup analysis was not included in the calculation (Figure 1b). The whole cohort PFS analyses are shown in Table IV. The statistically significant analyses for the whole cohort were also performed for the two treatment-associated subgroups and are shown in Table V.

Overall survival. For the whole CLL cohort, the median OS from the start of bendamustine-based therapy was 31.8 months. OS was influenced by several parameters and most importantly by the Rai stage (p=0.007), FISH karyotype risk-group (p<0.001), type of bendamustine therapy (p<0.001) and quality of response (p<0.001). In detail, the median OS was not reached for the cohort with 13q deletion only and the cohort with 17p deletion had the shortest median OS by far (8.3 months). As expected, the classification into patients with

Table I. *Patient characteristics of all patients with chronic lymphocytic leukemia (CLL) divided into bendamustine-rituximab therapy cohort and bendamustine-monotherapy cohort.*

Characteristic	All patients		Bendamustine+rituximab		Bendamustine	
	n (%)		n (%)		n (%)	
CLL, whole cohort	100		76		24	
Female	36 (36)		30 (40)		6 (25)	
Median age at initial diagnosis, median (range), years	98	66 (29-86)	74	66 (29-86)	24	68 (51-80)
Median time from diagnosis to bendamustine (range), months	98	75 (0.6-440)	74	75 (0.6-440)	24	73 (24-204)
Median age at start of bendamustine-based therapy, median (range)	100	73 (41-88)	76	73 (41-88)	24	74 (60-86)
CLL stage						
Rai I	11 (11)		9 (12)		2 (8)	
Rai II	22 (22)		19 (25)		3 (13)	
Rai III	27 (27)		22 (29)		5 (21)	
Rai IV	40 (40)		26 (34)		14 (58)	
Bendamustine line of therapy						
First	24 (24)		21 (28)		3 (13)	
Second	27 (27)		24 (32)		3 (13)	
Third or higher	49 (49)		31 (40)		18 (75)	
Prior treatment with fludarabine	43 (43)		30 (40)		13 (54)	
Fludarabine-refractory	7 (16)		5 (17)		2 (15)	
Median haemoglobin (range), g/dl	99	10.8 (5.5-15.8)	75	10.8 (6.5-15.8)	24	10.4 (5.5-15.3)
Median thrombocyte count (range), $\times 10^9$/l	99	110 (20-297)	75	134 (20-297)	24	91 (20-286)
Median β2-microglobulin (range), mg/l	62	4.6 (1.2-15.3)	51	4.4 (1.2-15.3)	11	5.6 (2.1-13.4)
Median LDH (range), U/l	87	259 (140-3162)	66	258 (146-696)	21	260 (140-3162)
Median GFR, ml/min	38	63 (27-100)	33	69 (27-100)	5	54 (41-64)
FISH karyotype						
13q deletion	20 (25)		16 (25)		4 (24)	
Normal cytogenetics	14 (17)		13 (20)		1 (6)	
Trisomy 12	12 (15)		12 (19)		0 (0)	
11q deletion	15 (19)		12 (19)		3 (18)	
17p deletion	20 (25)		11 (17)		9 (53)	
Bulky disease						
Yes	44 (50)		33 (48)		11 (55)	
No	44 (50)		35 (52)		9 (45)	
CIRS						
≤6	9 (22)		8 (23)		1 (17)	
>6	32 (78)		27 (77)		5 (83)	
ECOG status						
0-1	81 (87)		64 (90)		17 (77)	
2	12 (13)		7 (10)		5 (23)	
B-Symptoms	36 (39)		28 (40)		8 (36)	
No. of bendamustine applications, median (range)	100	8 (2-12)	76	10 (2-12)	24	7 (2-12)

CIRS, Cumulative illness rating scale; CLL, chronic lymphocytic leukemia; FISH, fluorescence *in situ* hybridization; GFR, glomerular filtration rate; LDH, lactate dehydrogenase.

good risk (13q deletion, normal karyotype, trisomy 12) and poor risk (11q deletion and 17p deletion) cytogenetics proved to be of major prognostic importance (median OS not reached *vs*. 14.3 months, $p<0.001$). Patients who reached CR after a bendamustine-based therapy had the longest median OS (median not reached), when compared to the other categories. Those with PR and SD had similar outcomes (OS=35.8 and 31.8 months, respectively), whereas PD was associated with a

Table II. *Overall response rates (ORR) for all patients treated with bendamustine-monotherapy or bendamustine-rituximab therapy.*

Characteristic	n	CR (%)	PR (%)	SD (%)	PD (%)	NE (%)	ORR (%)	*p*-Value
All patients	100	20 (20)	50 (50)	6 (6)	11 (11)	13 (13)	70 (70)	
Type of therapy								
Bendamustine+rituximab	76	17 (22)	41 (54)	4 (5)	6 (8)	8 (11)	58 (76)	0.014
Bendamustine	24	3 (13)	9 (38)	2 (8)	5 (21)	5 (21)	12 (50)	
Sex								
Male	64	11 (17)	31 (48)	6 (9)	8 (13)	8 (13)	42 (66)	0.203
Female	36	9 (25)	19 (53)	0 (0)	3 (8)	5 (14)	28 (78)	
Median age at start of bendamustine-based therapy								
<75 years	57	10 (18)	26 (46)	4 (7)	8 (14)	9 (16)	36 (63)	0.086
≥75 years	43	10 (23)	24 (56)	2 (5)	3 (7)	4 (9)	34 (79)	
CLL stage								
Rai I-II	33	6 (18)	23 (70)	0 (0)	3 (9)	1 (3)	29 (88)	0.006
Rai III-IV	67	14 (21)	27 (40)	6 (9)	8 (12)	12 (18)	41 (61)	
FISH karyotype								
13qDeletion	20	9 (45)	7 (35)	0 (0)	1 (5)	3 (15)	16 (80)	0.003
Normal cytogenetics	14	3 (21)	10 (71)	0 (0)	1 (7)	0 (0)	13 (93)	
Trisomy 12	12	1 (8)	8 (67)	2 (17)	1 (8)	0 (0)	9 (75)	
11q Deletion	15	1 (7)	7 (47)	2 (13)	3 (20)	2 (13)	8 (53)	
17p Deletion	20	1 (5)	6 (30)	2 (10)	5 (25)	6 (30)	7 (35)	
Therapy line								
First	24	5 (21)	14 (58)	1 (4)	2 (8)	2 (8)	19 (79)	0.261
Second or higher	76	15 (20)	36 (47)	5 (7)	9 (12)	11 (15)	51 (77)	
Prior fludarabine								
Yes	43	9 (21)	18 (42)	4 (9)	5 (12)	7 (16)	27 (63)	0.172
No	57	11 (19)	32 (56)	2 (4)	6 (11)	6 (11)	43 (75)	
Fludarabine-refractory								
Yes	7	0 (0)	3 (43)	1 (14)	3 (43)	0 (0)	3 (43)	0.280
No	7	2 (29)	3 (43)	0 (0)	0 (0)	2 (29)	5 (72)	
β2-Microglobulin								
≥2.4 mg/l	55	10 (18)	23 (42)	6 (11)	9 (16)	7 (13)	33 (60)	0.037
<2.4 mg/l	7	6 (86)	1 (14)	0 (0)	0 (0)	0 (0)	7 (100)	
LDH								
≥240 U/l	50	5 (10)	27 (54)	2 (4)	7 (14)	9 (18)	32 (64)	0.147
<240 U/l	37	12 (32)	17 (46)	4 (11)	3 (8)	1 (3)	29 (78)	
GFR								
≥70 ml/min	16	3 (19)	7 (44)	3 (19)	2 (13)	1 (6)	10 (63)	0.943
<70 ml/min	22	4 (18)	10 (46)	1 (5)	3 (14)	4 (18)	14 (64)	
No. of bendamustine applications								
≤8	55	5 (9)	25 (46)	3 (6)	9 (16)	13 (24)	30 (55)	<0.001
>8	45	15 (33)	25 (56)	4 (7)	2 (4)	0 (0)	45 (89)	
Cumulative dose								
<760 mg/m^2	50	3 (6)	23 (46)	4 (8)	7 (14)	13 (26)	26 (52)	<0.001
≥760 mg/m^2	50	17 (34)	27 (54)	2 (4)	4 (8)	0 (0)	44 (88)	
CIRS								
≤6	9	1 (11)	4 (44)	2 (22)	2 (22)	0 (0)	5 (56)	0.580
>6	32	6 (19)	15 (47)	2 (6)	4 (13)	5 (16)	21 (66)	

B, Bendamustine monotherapy; BR, bendamustine+rituximab; CIRS, cumulative illness rating scale; CR, complete response; FISH, fluorescence *in situ* hybridization; GFR, glomerular filtration rate; LDH, lactate dehydrogenase; NE, not evaluable; ORR; overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

Table III. *Overall response rates (ORR) for significant factors in the total cohort analysis, comparing bendamustine-rituximab therapy cohort to bendamustine-monotherapy cohort.*

Characteristics	Bendamustine+rituximab				Bendamustine			
	n	ORR (%)	NE (%)	*p*-Value	n	ORR (%)	NE (%)	*p*-Value
CLL stage								
Rai I-II	28	25 (89)	1 (4)	0.042	5	4 (80)	0 (0)	0.132
Rai III-IV	48	33 (69)	7 (15)		19	8 (42)	5 (26)	
FISH karyotype								
13q Deletion	16	14 (88)	2 (13)	0.043	4	2 (50)	1 (25)	0.409
Normal cytogenetics	13	12 (92)	0 (0)		1	1 (100)	0 (0)	
Trisomy 12	12	9 (75)	0 (0)		0	0 (0)	0 (0)	
11q Deletion	12	7 (58)	1 (8)		3	1 (33)	1 (33)	
17p Deletion	11	5 (46)	4 (36)		9	2 (22)	2 (22)	
β2-Microglobulin								
≥2.4 mg/l	45	31 (69)	4 (9)	0.132	10	2 (20)	3 (30)	0.038
<2.4 mg/l	6	6 (100)	0 (0)		1	1 (100)	0 (0)	
No. of bendamustine applications								
≤8	36	22 (61)	8 (22)	0.003	19	8 (42)	5 (26)	0.127
>8	40	40 (90)	0 (0)		5	4 (80)	0 (0)	
Cumulative bendamustine dose								
<760 mg/m^2	33	20 (61)	8 (24)	0.005	17	6 (35)	5 (29)	0.025
≥760 mg/m^2	43	38 (88)	0 (0)		7	6 (86)	0 (0)	
CIRS								
≤6	8	4 (50)	0 (0)	0.127	1	1 (100)	0 (0)	0.050
>6	27	21 (78)	3 (11)		5	0 (0)	2 (40)	

CIRS, Cumulative illness rating scale; FISH, fluorescence *in situ* hybridization; NE, not evaluable; ORR; overall response rate.

significantly shorter median OS (9.2 months; $p<0.001$). In general, the BR cohort (n=76) was characterized by a superior median OS compared to the bendamustine-treated cohort (n=24; OS=42.7 months *vs*. 14.3 months; $p<0.001$; Figure 2a). The median OS of the subgroup of treatment-naïve patients (n=24; median OS not reached) was significantly superior to that of the pre-treated patients (n=76; OS=29.0 months; $p=0.027$). Furthermore, when analyzing solely the BR group, consisting of 76 patients, the treatment-naïve subgroup (n=21) had a significantly longer median OS compared to pre-treated patients (n=55) (not reached *vs*. 35.8 months; $p=0.050$; Figure 2b). The complete analysis is shown in Table IV and V.

Multivariate analysis was performed for the whole cohort, and in the first model, only pre-treatment baseline characteristics that showed significance in the univariate analysis were included (n=81). Parameters independently influencing OS were lower Rai stage ($p=0.030$) and good-risk cytogenetics ($p<0.001$) (not shown in detail). The second model of multivariate analysis included pre-treatment baseline characteristics which were significant in the first multivariate analysis and treatment-related parameters that were significant in the univariate analysis (n=81) (shown in Table VI). The independent parameters associated with longer median OS were lower Rai stage ($p=0.020$); good-risk cytogenetics ($p<0.001$); administration of BR ($p=0.009$); and response to treatment ($p=0.026$).

Toxicity. All observed adverse events occurring from the beginning of bendamustine-based therapy until two months after the last administration were classified according to the CTC criteria version 4. Hematological and non-hematological adverse advents are detailed in Table VII. Briefly, grade 3/4 hematological adverse events were leukopenia in 26/91 patients (29%), neutropenia in 26/90 (29%), anemia in 14/94 (15%), and thrombocytopenia in 17/95 patients (18%). Febrile neutropenia was observed in 4/70 cases (6%). Among non-hematological toxicities, severe infections of grades 3/4 were reported in 21 out of 70 cases (30%), two of which were fatal. Nine patients died during bendamustine therapy or within 2 months after the last dose

Table IV. *Progression free survival (PFS) and overall survival (OS) for all patients treated with bendamustine-monotherapy or bendamustine-rituximab therapy.*

Characteristic	n	Median PFS, months	*p*-Value	Median OS, months	*p*-Value
All patients	100	17.7		31.8	
Type of therapy					
Bendamustine	24	8.3	<0.001	14.3	<0.001
Bendamustine+rituximab	76	22.5		42.7	
Sex					
Male	64	13.5	0.266	29	0.412
Female	36	22.5		n.r.	
Median age at start of bendamustine-based therapy					
<75 years	57	11.7	0.237	35.8	0.779
≥75 years	43	23.4		29.0	
B-CLL stage					
Rai I-II	33	22.5	0.061	n.r.	0.007
Rai III-IV	67	11.6		25.6	
Response					
CR	20	n.r.	<0.001	n.r.	<0.001
PR	50	18.9		35.8	
SD	6	11.6		31.8	
PD	11	3.0		9.2	
NE	13	2.1		2.7	
FISH karyotype					
13q Deletion	20	31.7	<0.001	n.r.	<0.001
Normal karyotype	14	22.5		42.7	
Trisomy 12	12	27.3		31.8	
11q Deletion	15	9.3		21	
17p Deletion	20	4.4		8.3	
Therapy line					
First	24	27.3	0.054	n.r.	0.027
Second or higher	76	12.1		29.0	
Prior fludarabine					
Yes	43	10.4	0.072	29.0	0.316
No	57	22.5		42.7	
Fludarabine-refractory					
Yes	7	9.1	0.161	21.5	0.056
No	7	31.7		n.r.	
β2-Microglobulin					
≥2.4 mg/l	55	11.6	0.153	31.8	0.490
<2.4 mg/l	7	n.r.		n.r.	
GFR					
≥70 ml/min	16	17.7	0.314	42.7	0.186
<70 ml/min	22	10.7		31.8	
Cumulative bendamustine dose					
<760 mg/m^2	50	10.4	0.005	19.9	0.068
≥760 mg/m^2	50	23.4		35.8	
CIRS					
≤6	9	9.1	0.857	42.6	0.492
>6	32	12.1		35.8	

CIRS, Cumulative illness rating scale; CLL, chronic lymphocytic leukemia; CR, complete response; FISH, fluorescence *in situ* hybridization; GFR, glomerular filtration rate; n.r., not reached; NE, not evaluable; ORR; overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

Table V. *Progression free survival (PFS) and overall survival (OS) for significant factors total cohort analysis, comparing bendamustine-rituximab therapy cohort to the bendamustine-monotherapy cohort.*

Characteristics	Bendamustine+rituximab					Bendamustine				
	n	Median PFS, months	*p*-Value	Median OS, months	*p*-Value	n	Median PFS, months	*p*-Value	Median OS, months	*p*-Value
All patients	76	22.5		42.7		24	8.3		14.3	
B-CLL stage										
Rai I-II	28	29	0.141	n.r.	0.043	5	13.5	0.431	30.9	0.129
Rai III-IV	48	12.3		31.8		19	4.4		9.2	
Response										
CR	17	n.r.	<0.001	n.r.	0.008	3	8.3	<0.001	8.3	0.001
PR	41	22.5		n.r.		9	13.5		25.6	
SD	4	10.7		31.8		2	n.r.		n.r.	
PD	6	4.5		42.7		5	1.7		7.2	
NE	8	3.1		8.3		5	0.8		0.8	
FISH karyotype										
13q Deletion	16	31.7	<0.001	n.r.	<0.001	4	3.0	0.391	n.r.	0.249
Normal karyotype	13	22.5		n.r.		1	15.3		n.r.	
Trisomy 12	12	27.3		n.r.		0	NE		NE	
11q Deletion	12	11.6		25.6		3	2.0		12.6	
17p Deletion	11	5.7		9.3		9	1.7		5.8	
Therapy line										
First	21	27.3	0.120	n.r.	0.050	3	1.7	0.932	7.2	0.687
Second or higher	55	17.7		35.8		21	8.3		14.3	
Cumulative bendamustine dose										
<760 mg/m^2	33	12.1	0.024	n.r.	0.273	17	2.0	0.501	12.6	0.533
≥760 mg/m^2	43	25.7		42.7		7	11.7		14.3	

CR, Complete response; FISH, fluorescence *in situ* hybridization; n.r., not reached; NE, not evaluable; PD, progressive disease; PFS, progression free survival; PR, partial response; OS, overall survival; SD, stable disease.

due to sepsis (n=2), rapid CLL progression (n=2), or unknown causes (n=5). Secondary malignancies, during or after therapy, occurred in five cases (peritoneal cancer of unknown primary; melanoma; gastric cancer; rectal cancer; myelodysplasia). Of note, interstitial pulmonary infiltrates considered potentially therapy-associated occurred only in patients receiving the BR combination treatment (n=4, 6%). We created sub-groups to check for adverse events (grade 3 or 4 leukocytopenia, neutropenia, anemia, thrombocytopenia, and infection) by bendamustine-based therapy in different settings, defined by age, gender, prior treatment (none *vs.* any), prior fludarabine, fludarabine refractoriness, CIRS score (>6 *vs.* ≤6), GFR (≥70 *vs.* <70 ml/min), bulky disease and cytogenetic risk category. No significant results in these sub-group analyses (not shown in detail) were found. Furthermore, no significant difference in toxicity between the BR and bendamustine-treated cohorts was found in these subgroup analyses.

Discussion

CLL is a lymphoproliferative disease occurring mostly in patients with advanced age and therefore the treatment decisions are essentially influenced by the comorbidities of the patients (4, 6, 23, 24). We aimed to study the use of bendamustine in a real-life CLL patient cohort in Austria and Italy. The patient cohort analyzed is well-balanced and reflects a real-world CLL cohort, with patients mostly in higher-therapy lines and with comorbidities (median CIRS score=9, median GFR=63 ml/min). In total, 76% of the patients were treated with bendamustine in combination with rituximab; 24% of the patients were treated with bendamustine monotherapy.

Another aim of this study was to evaluate patient response and survival, especially comparing patients who received BR and those who received bendamustine monotherapy. The ORR was in line with those of other studies, not only in the

Table VI. *Multivariate analysis of overall survival (OS) of the total cohort treated with bendamustine-monotherapy or bendamustine-rituximab therapy.*

Parameter	Univariate analysis	Multivariate analysis	
	p-Value	*p*-Value	Hazard ratio (95% confidence interval)
Chemonaïve	0.027	0.418	0.639 (0.216-1.889)
CLL stage (Rai I+II)	0.007	0.020	0.249 (0.077-0.803)
Good-risk cytogenetics (13q deletion, normal karyotype, trisomy 12)	<0.001	<0.001	0.134 (0.047-0.377)
Type of bendamustine therapy (BR)	<0.001	0.009	0.318 (0.135-0.747)
Response (CR+PR)	<0.001	0.026	0.408 (0.185-0.900)

BR, Bendamustine+rituximab; CLL, chronic lymphocytic leukemia; CR, complete response; PR, partial response.

Table VII. *Toxicities of all patients treated with bendamustine-monotherapy or bendamustine-rituximab therapy.*

Adverse event	n	Grade III (%)	Grade IV (%)
Leukopenia	91	17 (19)	9 (10)
Neutropenia	90	16 (18)	10 (11)
Anemia	94	9 (10)	5 (5)
Thrombocytopenia	95	9 (10)	8 (8)
Infection	70	13 (19)	8 (11)
Skin toxicity	68	5 (7)	0 (0)
Nausea	68	1 (2)	0 (0)
Paresthesia	67	0 (0)	0 (0)
Hypersensitivity reaction	67	0 (0)	0 (0)
Febrile neutropenia	70	n.a.	4 (6)
Interstitial pneumonitis/chronic organizing pneumonia	67	4 (6)	0 (0)

n.a., Not applicable.

overall cohort, but also in the two sub-groups of patients which received BR or solely bendamustine. Of note, patients undergoing treatment with BR achieved significantly higher ORR (76%) and CR (22%) rates than those receiving single-agent bendamustine (ORR and CR of 50% and 13%, respectively). PFS and OS were also significantly lower in the single-agent bendamustine-treated group, with OS of 14.3 months and PFS of 8.3 months compared to the BR group with OS of 42.7 and PFS of 22.5 months (both $p<0.001$) (Figure 1 and 2). The results suggest that bendamustine should no longer be used as monotherapy in CLL, but that it has significant value as a chemotherapy backbone when combined with rituximab in older, less-fit patients with CLL, especially when considering that no significant differences in treatment-related toxicities were found. However, when comparing these sub-groups, we should bear in mind that the bendamustine monotherapy cohort included more high-risk patients in terms of cytogenetics, Rai stage and median number of previous therapies (shown in Table I).

No prospective study comparing bendamustine monotherapy and the BR combination therapy has yet been conducted in CLL and probably never will be. However, recognizing the epidemiological imbalances mentioned above as well as the limitations of a retrospective study, the data presented here are in line with the improved outcomes observed in prospective first- (25, 26) and second-line trials (27). Hence, the addition of rituximab to bendamustine may have a similar positive impact on patient response in CLL as seen in other immunochemotherapy studies including purine analogs with/without cyclophosphamide as chemotherapy backbone. Other retrospective studies showed similar responses, whereas the prospective studies have a noticeable better response (ORR=98%, with CR=38%) (2, 13). Moreover, a relatively small sub-group of 21 patients received BR in the first-line setting and the treatment combination proved to be very effective in this setting (Figure 1b and 2b).

Interestingly, a CIRS score over 6, GFR less than 60 ml/min, and patient age greater than 75 years were not unfavorable in ORR, PFS, and OS. Although chemotherapy with fludarabine, cyclophosphamide and rituximab (FCR) is currently considered to be the most active therapeutic regimen in CLL (26), it is associated with considerable toxicity and is often too toxic for older patient populations. Therefore, we await the final results of the CLL-10 study comparing BR and FCR in fit patients with CLL (28).

The applied cumulative dose of bendamustine (>750 mg/m^2) seems to be an important prognostic factor. In our study, dose application was no problem and in general bendamustine was well-tolerated (median number of applied cycles in the BR group was 5). The most common grade 3 and 4 toxicities were hematological, with leukopenia (29%) and neutropenia (29%) being the most frequent. Anemia (15%) and thrombocytopenia (18%) were less common therapy-associated toxicities. Non-hematological toxicities were infections (30%) followed by skin toxicities (7%) and nausea (2%). The observed toxicities are common in bendamustine treatment and are in line with those already reported (2, 12, 29). Interestingly, toxicities did not occur more often in older patients, those with fludarabine-refractory disease, or those with a high CIRS score, or low GFR.

In conclusion, we describe an unselected population of patients with CLL (elderly patients, higher lines of therapy and mostly fludarabine pre-treated, with impaired renal function) in which bendamustine demonstrates a favorable efficacy and toxicity profile in routine clinical use.

Conflicts of Interest

MN, AP, MM, PM, FK, CW, and IV have no conflict of interest to disclose. MF received a grant for this observational study; MF, MS and GG received honoraria from Mundipharma Austria GmbH for lectures. MM received honoraria from Mundipharma for participation in an advisory board.

Acknowledgements

This study was supported by the Verein für Tumorforschung, Innsbruck. We thank all centers participating and documenting patients for the study.

References

1 Knauf WU, Lissichkov T, Aldaoud A, Liberati A, Loscertales J, Herbrecht R, Juliusson G, Postner G, Gercheva L, Goranov S, Becker M, Fricke HJ, Huguet F, Del G, I, Klein P, Tremmel L, Merkle K, and Montillo M: Phase III randomized study of bendamustine compared with chlorambucil in previously untreated patients with chronic lymphocytic leukemia. J Clin Oncol *27*: 4378-4384, 2009.

2 Kolibaba KS, Sterchele JA, Joshi AD, Forsyth M, Alwon E, Beygi H, and Kennealey GT: Demographics, treatment patterns, safety, and real-world effectiveness in patients aged 70 years and over with chronic lymphocytic leukemia receiving bendamustine with or without rituximab: a retrospective study. Ther Adv Hematol *4*: 157-171, 2013.

3 Byrd JC: Chronic lymphocytic leukemia: state of the art and beyond. J Natl Compr Canc Netw 12: 801-803, 2014.

4 Byrd JC, Jones JJ, Woyach JA, Johnson AJ, and Flynn JM: Entering the era of targeted therapy for chronic lymphocytic leukemia: impact on the practicing clinician. J Clin Oncol *27*: 3039-47, 2014.

5 Eichhorst B, Hallek M, and Dreyling M: Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 21 Suppl 5: v162-v164, 2010.

6 Hallek M: Chronic lymphocytic leukemia: 2013 update on diagnosis, risk stratification and treatment. Am J Hematol *88*: 803-816, 2013.

7 Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, and Kipps TJ: Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood *111*: 5446-5456, 2008.

8 Dreger P, Corradini P, Kimby E, Michallet M, Milligan D, Schetelig J, Wiktor-Jedrzejczak W, Niederwieser D, Hallek M, and Montserrat E: Indications for allogeneic stem cell transplantation in chronic lymphocytic leukemia: the EBMT transplant consensus. Leukemia *21*: 12-17, 2007.

9 Rummel MJ, Niederle N, Maschmeyer G, Banat GA, von GU, Losem C, Kofahl-Krause D, Heil G, Welslau M, Balser C, Kaiser U, Weidmann E, Durk H, Ballo H, Stauch M, Roller F, Barth J, Hoelzer D, Hinke A, and Brugger W: Bendamustine plus rituximab *versus* CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase III non-inferiority trial. Lancet *381*: 1203-1210, 2013.

10 Hallek M: State-of-the-art treatment of chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program 440-449, 2009.

11 Cheson BD, Wendtner CM, Pieper A, Dreyling M, Friedberg J, Hoelzer D, Moreau P, Gribben J, Knop S, Montillo M, and Rummel M: Optimal use of bendamustine in chronic lymphocytic leukemia, non-Hodgkin lymphomas, and multiple myeloma: treatment recommendations from an international consensus panel. Clin Lymphoma Myeloma Leuk *10*: 21-27, 2010.

12 Fischer K, Cramer P, Busch R, Bottcher S, Bahlo J, Schubert J, Pfluger KH, Schott S, Goede V, Isfort S, von TJ, Fink AM, Buhler A, Winkler D, Kreuzer KA, Staib P, Ritgen M, Kneba M, Dohner H, Eichhorst BF, Hallek M, Stilgenbauer S, and Wendtner CM: Bendamustine in combination with rituximab for previously untreated patients with chronic lymphocytic leukemia: a multicenter phase II trial of the German Chronic Lymphocytic Leukemia Study Group. J Clin Oncol *30*: 3209-3216, 2012.

13 Knauf W, Abenhardt W, Dorfel S, Meyer D, Grugel R, Munz M, Hartmann H, and Marschner N: Routine treatment of patients with chronic lymphocytic leukaemia by office-based haematologists in Germany-data from the Prospective Tumour Registry Lymphatic Neoplasms. Hematol Oncol *1*:15-22, 2015.

14 NCCN Guidelines CLL/SLL, last update 2014. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp

15 Fiegl M, Stauder R, Steurer M, Mian M, Hopfinger G, Brychtova Y, Skrabs C, Zabernigg A, Schmid F, Haslbaur F, Winder G, Walder A, Lang A, Voskova D, Greil R, Mayer J, and Gastl G: Alemtuzumab in chronic lymphocytic leukemia: final results of a large observational multicenter study in mostly pretreated patients. Ann Hematol *93*: 267-277, 2014.

16 Waldthaler C, Stauder R, Schnallinger M, Schreieck S, Hager J, Oexle H, Zangerl G, Verdorfer I, Zabernigg A, Gastl G, and Fiegl M: Routine use of bendamustine and rituximab combination therapy in consecutive patients with lymphoproliferative diseases: a survey from Tyrolean hospitals. Wien Klin Wochenschr *123*: 269-275, 2011.

17 Fiegl M, Erdel M, Tinhofer I, Brychtova Y, Panovska A, Doubek M, Eigenberger K, Fonatsch C, Hopfinger G, Muhlberger H, Zabernigg A, Falkner F, Gastl G, Mayer J, and Greil R: Clinical outcome of pretreated B-cell chronic lymphocytic leukemia following alemtuzumab therapy: a retrospective study on various cytogenetic risk categories. Ann Oncol *21*: 2410-2419, 2010.

18 Zaja F, Mian M, Volpetti S, Visco C, Sissa C, Nichele I, Castelli M, Ambrosetti A, Puglisi S, Fanin R, Cortelazzo S, Pizzolo G, Trentin L, Rodeghiero F, Paolini R, Vivaldi P, Sancetta R, Isola M, and Semenzato G: Bendamustine in chronic lymphocytic leukemia: outcome according to different clinical and biological prognostic factors in the everyday clinical practice. Am J Hematol *88*: 955-960, 2013.

19 US Department of Health, National Cancer Institut. Common Terminology Criteria for Adverse Events v4.0 (CTCAE). NIH Publication No.09-5410 . 1-5-2009.

20 Linn BS, Linn MW, and Gurel L: Cumulative illness rating scale. J Am Geriatr Soc *16*: 622-626, 1968.

21 Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, and Lichter P: Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med *343*: 1910-1916, 2000.

22 Keating MJ, O'Brien S, Kontoyiannis D, Plunkett W, Koller C, Beran M, Lerner S, and Kantarjian H: Results of first salvage therapy for patients refractory to a fludarabine regimen in chronic lymphocytic leukemia. Leuk Lymphoma *43*: 1755-1762, 2002.
23 Hallek M and Pflug N: State of the art treatment of chronic lymphocytic leukaemia. Blood Rev *25*: 1-9, 2011.
24 Eichhorst B, Goede V, and Hallek M: Treatment of elderly patients with chronic lymphocytic leukemia. Leuk Lymphoma *50*: 171-178, 2009.
25 Byrd JC, Rai K, Peterson BL, Appelbaum FR, Morrison VA, Kolitz JE, Shepherd L, Hines JD, Schiffer CA, and Larson RA: Addition of rituximab to fludarabine may prolong progression-free survival and overall survival in patients with previously untreated chronic lymphocytic leukemia: an updated retrospective comparative analysis of CALGB 9712 and CALGB 9011. Blood *105*: 49-53, 2005.
26 Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, Hensel M, Hopfinger G, Hess G, von GU, Bergmann M, Catalano J, Zinzani PL, Caligaris-Cappio F, Seymour JF, Berrebi A, Jager U, Cazin B, Trneny M, Westermann A, Wendtner CM, Eichhorst BF, Staib P, Buhler A, Winkler D, Zenz T, Bottcher S, Ritgen M, Mendila M, Kneba M, Dohner H, and Stilgenbauer S: Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet *376*: 1164-1174, 2010.
27 Robak T, Dmoszynska A, Solal-Celigny P, Warzocha K, Loscertales J, Catalano J, Afanasiev BV, Larratt L, Geisler CH, Montillo M, Zyuzgin I, Ganly PS, Dartigeas C, Rosta A, Maurer J, Mendila M, Saville MW, Valente N, Wenger MK, and Moiseev SI: Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol *28*: 1756-1765, 2010.
28 Eichhorst B, Fink AM, Busch R, Lange E, Köppler H, Kiehl MG, Sökler M, Schlag R, Vehling-Kaiser U, Köchling G, Plöger C, Gregor M, Plesner T, Trneny M, Fischer K, Göhner H, Kneba M, Wendtner C, Klapper W, Kreuzer KA, Stilgenbauer S, Böttcher S, Hallek M: Chemoimmunotherapy with fludarabine (F), cyclophosphamide (C), and rituximab (R) (FCR) *versus* bendamustine and rituximab (BR) in previously untreated and physically fit patients (pts) with advanced chronic lymphocytic leukemia (CLL): Results of a planned interim analysis of the CLL10 trial. Blood Proc ASH: Abstract *526*: 2013.
29 Fischer K, Cramer P, Busch R, Stilgenbauer S, Bahlo J, Schweighofer CD, Bottcher S, Staib P, Kiehl M, Eckart MJ, Kranz G, Goede V, Elter T, Buhler A, Winkler D, Kneba M, Dohner H, Eichhorst BF, Hallek M, and Wendtner CM: 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. J Clin Oncol *29*: 3559-3566, 2011.

Received April 17, 2015
Revised May 19, 2015
Accepted May 22, 2015