ARTICLE

Efficacy of bendamustine and rituximab as first salvage treatment in chronic lymphocytic leukemia and indirect comparison with ibrutinib: a GIMEMA, ERIC and UK CLL FORUM study

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ABSTRACT

We performed an observational study on the efficacy of bendamustine and rituximab (BR) as first salvage regimen in chronic lymphocytic leukemia (CLL). In an intention-to-treat analysis including 237 patients, the median progression-free survival (PFS) was 25 months. The presence of del(17p), unmutated IGHV and advanced stage were associated with a shorter PFS at multivariate analysis. The

median time-to-next treatment was 31.3 months. Front-line treatment with a chemoimmunotherapy regimen was the only predictive factor for a shorter time to next treatment at multivariate analysis. The median overall survival (OS) was 74.5 months. Advanced disease stage (i.e. Rai stage III-IV or Binet stage C) and resistant disease were the only parameters significantly associated with a shorter OS. Grade 3-5 infections were recorded in 6.3% of patients. A matched-adjusted indirect comparison with ibrutinib given second-line within Named Patient Programs in the United Kingdom and in Italy was carried out with OS as objective end point. When restricting the analysis to patients with intact 17p who had received chemoimmunotherapy in first line, there was no difference in OS between patients treated with ibrutinib (63% alive at 36 months) and patients treated with BR (74.4% alive at 36 months). BR is an efficacious first salvage regimen in CLL in a real-life population, including the elderly and unfit patients. BR and ibrutinib may be equally effective in terms of OS when used as first salvage treatment in patients without 17p deletion. (Registered at *clinicaltrials.gov identifier: 02491398*)

Introduction

Treatment of chronic lymphocytic leukemia (CLL) has dramatically changed over the last years. Chemotherapy and anti-CD20 monoclonal antibodies produce high overall response rates (ORR), including complete remissions (CR) with negative minimal residual disease, and prolonged progression-free-survival (PFS) and overall survival (OS), both in fit[1,2] and unfit patients.[3] In patients with *TP53* disruption and/or with relapsed/refractory (R/R) disease, who represent a difficult-to-treat patient population, mechanism-driven drugs targeting the Bruton tyrosine kinase (BTK), the phosphoinositide 3-kinase delta (PI3K δ) or the BCL2 protein can induce durable responses.[4-7]

In the absence of *TP53* disruption, a chemoimmunotherapy (CIT) regimen is recommended as front-line and second-line treatment in those patients who attained a long progression-free survival (PFS) with the previous regimen.[8,9] On the other hand, the National Comprehensive Cancer Network recommends one of the new agents, ibrutinib, idelalisib with rituximab or venetoclax, as alternatives to CIT for patients with relapsed or refractory disease.[10]

Uncertainty in the recommendations on first salvage treatment may partly derive from the consideration that the majority of studies on R/R CLL report efficacy data in an aggregate fashion, analyzing patients who had previously received one or more lines of treatment all together. Consequently, little information is currently available on the outcome of second-line treatment.

Bendamustine and rituximab (BR) is one of the most widely adopted CIT regimens, both as front-line[11] and second-line treatment.[12-14] The BR regimen was followed by a median PFS of 18 months when used as first salvage treatment after fludarabine, cyclophosphamide and rituximab (FCR) in 62 patients regardless of TP53 aberrations and/or refractoriness to prior therapy.[13] In 78 CLL patients who had received 1-3 previous lines of treatment, the BR combination was associated with a 59% ORR with a median PFS of 15.2-months.[15] Bendamustine and ofatumumab produced a 23.6-month median PFS in 47 patients, 61% and 29% of whom had received 1 or 2 prior lines of treatment, respectively.[16]

The oral agent ibrutinib represents an effective therapy in the R/R setting.[17] In a recent analysis describing a 5-year experience, a median PFS of 52 months was reported in R/R CLL treated with ibrutinib after 4 or more previous lines of treatment in more than 50% of patients.[18] In a recent update of the phase III Resonate study comparing ibrutinib and ofatumumab, the PFS rate appeared to be better in patients treated with ibrutinib in second line compared to patients who had received 2 or more previous lines of treatment.[19]

Recent experiences with ibrutinib in a real-world setting have reported a higher rate of discontinuation compared to clinical trials,[20,21] possibly due to older age and worse Performance Status (PS) of the patient population treated in the day-to-day clinical practice.[22]

On these grounds, we performed a retrospective observational study within the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) and European Research Initiative on CLL (ERIC) networks to collect data on the efficacy and safety profile of the BR regimen used as second-line treatment in a real-world setting. We then set out to perform an indirect comparison with ibrutinib given as first salvage treatment in the UK and the Italian Named Patient Programs (NPP).

Methods

Patients

Patients treated between 2008 and 2014 at GIMEMA and ERIC centers were eligible. The inclusion criteria were: i) diagnosis of CLL according to the National Cancer Institute (NCI);[23] ii) age ≥18 years; iii) one previous treatment using alkylating agents and/or purine analogs with or without monoclonal antibodies; iv) progression requiring therapy (NCI criteria);[23] v) second-line treatment with BR at the conventional dose of 70 mg/m^2, as described.[15]

Patients were excluded if they had Richter's syndrome transformation, HIV infection, active HCV or HBV infection. The study was registered at *clinicaltrials.gov identifier: 02491398*. The study was approved by the local ethics committees.

Study design and end points

Data were obtained from the medical files and entered into case record forms (CRF) by treating physicians. Computerized and manual consistency checks were performed by the data manager of the GIMEMA data center.

Evaluation of bone marrow response and radiographic imaging at baseline and at response were performed according to local guidelines. Treatment response and disease progression were assessed according to the NCI criteria.[23]

Primary end point

The primary end point was PFS at 12 months from treatment start. Subjects who were withdrawn from the study without progression were censored at the date of the last assessment. Subjects

without post-baseline assessments but known to be alive were censored at the time of first dose of study drug.

Secondary end points

The ORR was assessed in all the patients who started treatment (intention to treat). Time to next anti-leukemic treatment (TTNT) was calculated using the cumulative incidence method, from the date of the first dose of the study drugs until the date of retreatment. OS was calculated from the date of the first dose of the study drug until the date of death. Patients without follow-up assessment were censored at the day of the last treatment administration. Evaluation of safety was reported according to NCI Common Terminology Criteria for Adverse Events version 4.0.

Indirect comparison with ibrutinib

Data from patients treated in second line with single agent ibrutinib in the UK and Italy within the NPP were retrospectively retrieved. Patients with R/R CLL treated in the UK have been reported previously.[20] Patients treated in Italy were extracted from the GIMEMA LLC1415 trial (*clinicaltrials.gov identifier: 02582320*). The end point for this analysis was OS.

Statistical analysis

Statistical analysis was performed following the intention-to-treat principle. Non-parametric tests were applied for comparisons between groups (χ^2 and Fisher Exact test for categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test for continuous variables) and logistic regression were applied in multivariate analysis. Survival distributions were estimated using the Kaplan-Meier Product Limit estimator.

Differences in terms of PFS, TTNT and OS were evaluated by Log-Rank test in univariate analysis and Cox regression model in multivariate analysis.

Cumulative Incidence curves were estimated using the proper non-parametric method. The Gray test was applied for significance tests on cumulative incidence curves.

All the analyses were performed using the SAS software (v.9.4 or later); all tests were two-sided. P=0.05 was considered statistically significant. Confidence intervals were calculated at 95% (95%CI).

Results

Patients' characteristics

A total of 237 patients treated at 37 centers (28 centers belonging to the GIMEMA group and 9 centers affiliated with the ERIC group) were enrolled (*Online Supplementary Table S1*). Baseline patients' characteristics are outlined in Table 1: median age was 70.4 years, range 39.4-87.8; 70.9% of patients were over 65 years old; 58.3% had 2 or more comorbidities; 46.9% had a creatinine clearance ≤70 ml/min; and 21.4% had an advanced disease stage (i.e. Rai III-IV or Binet C). Seventy-three percent (data available in 61.6% of the patients) had an unmutated tumor immunoglobulin gene heavy chain variable region configuration (U-*IGHV*) and 33.4% had 11q- and/or 17p13 deletion (data available in 79.3% of the patients). These patients were representative of the entire study population in terms of baseline characteristics and outcome (*data not shown*).

Table 1. Patients' characteristics.

Variable	Frequency (%) Benda + R n=237	Ibrutinib n=95	*P*
Age, years [median, range]	70.4 [39.4-87.8]	69.3 [27.5-85.3]	0.344
Age, years ≤65/>65	69 (29.1)/168(70.9)	32 (34.0)/62 (66.0)	0.427
Sex, M/F	168 (70.9)/69 (29.1)	60 (63.2)/35 (36.8)	0.215
ECOG PS (%) 0-1/≥2	198 (90.0)/22 (10.0)	75 (83.3)/15 (16.7)	0.147
Stage, Rai III/IV or Binet C no/yes	165 (78.6)/45 (21.4)	–	–
Bulky lymph nodes (> 5cm) no/yes	20 (8.9)/204 (91.1)	–	–
Comorbidities 0-1/≥2	98 (41.7)/137 (58.3)	–	–
Creatinine clearance (mL/min) ≤ 70/>70	100 (46.9)/113 (53.1)	–	–
CD38 (>20%) neg/pos	52 (47.3)/58 (52.7)	–	–
17p- yes/no	23 (12.6)/ 160 (87.4)	33 (39.8)/ 50 (60.2)	<0.001
FISH 13q-/+12/11q-/17p-/no aberrations	45 (24.6)/32 (17.5)/38 (20.8)/23 (12.6)/45 (24.6)	–	–
IGHV Mutated/unmutated	40 (27.4)/106 (72.6)	14 (38.9)/22 (61.1)	0.251
Months between 1st and 2nd treatment <36/≥ 36	124 (52.3)/113 (47.7)	54 (75.0)/18 (25.0)	0.001
Previous treatment			–
ORR rate to 1st line treatment (%) yes/no	195 (82.3)/42 (17.7)	56 (78.9)/15 (21.1)	0.636
Refractory no/yes	174 (90.6)/18 (9.4)	–	–
CIT no/yes	95 (41.0)/137 (59.0)	22 (23.7)/71 (76.3)	0.005
Chemo Chl/FL-based/bendamustine	39 (41.1)/42 (44.2)/14 (14.7)	–	–
CIT Chl/F-based/bendamustine	19 (13.9)/77 (56.2)/41 (29.9)	–	–
<6 cycles and/or dose reductions yes/no	140 (59.6)/95 (40.4)	–	–

n: number; ECOG: Eastern Cooperative Oncology Group; neg: negative; pos: positive; ORR: overall response rates; chemo: chemotherapy; Chl: chlorambucil; CIT: chemoimmunotherapy; M: male; F: female; FL: fludarabine.

First-line treatment included CIT regimens combining rituximab with fludarabine (with or without cyclophosphamide), bendamustine or chlorambucil in 59% of patients; 41% of patients received chemotherapy or, in 2 cases, single agent treatment with rituximab or alemtuzumab. No patient received ibrutinib or other novel oral agents front line. The use of chemotherapy alone front line was more frequent before 2010 (52.8% of patients) than from 2011 onwards (27.9% of patients). Eighteen patients (9.4%) were refractory to first-line treatment.

Treatment with BR

One hundred and sixty-five of the 237 patients (69.6%) received the planned number of cycles; treatment was discontinued early in 72 patients as a result of toxicity (n=39), withdrawal of consent (n=7), progressive disease (n=6), or for other reasons (n=20). The number of cycles actually administered to patients who discontinued treatment was ≥4 in 52.8% (n=38) of cases.

Dose reduction of over 10% of the planned dose of bendamustine (i.e. <70 mg/m^2) was recorded in 28.9% of cases; a treatment delay occurred in 22.5% of patients. Overall, 95 patients (40.1%) received 6 cycles without dose reduction. The median dose administered to the patients who discontinued treatment or received a reduced dose was 350 mg/m^2.

Efficacy

The 12-month PFS rate was 78.6% (95%CI: 73.5-84.1%). The estimated PFS at 30 and 60 months was 30.9% (95%CI: 24.8-38.5%) and 16.2% (95%CI: 10.6-24.6%), respectively, with a median overall PFS of 25 months (Figure 1) (median follow up 37.1 months, range 0.4-98.5).

Factors predicting for a shorter PFS at univariate analysis (Table 2) were 17p deletion (median 14.5 months *vs.* 25.5 months), U-IGHV (median 20.7 months *vs.* 32.1) and a less than 36-month interval between first- and second-line treatment (median 21.1 months *vs.* 26.8), whereas an advanced stage was of borderline significance (median 20.6 *vs.* 25.8 months). Age (cutoff 65 years), creatinine clearance [cutoff 70 mL/minute (min)] and the presence or absence of 2 or more comorbidities had no impact on PFS. The presence of 17p-, U-IGHV and Binet/Rai stage C/III-IV were associated with a shorter PFS at multivariate analysis (Table 2). Patients with a low-risk profile, i.e. without del(17p), with M-IGHV and Rai stage 0-2 (12.2% of the total patient population), had a median PFS of 40.4 months compared to 20.7 months in the remaining patients (*P*=0.003) (*Online Supplementary Figure S1*).

The ORR was 82.3% and the probability of attaining a response was significantly lower in patients with del(17p) (69.6%) compared to patients with del(11q) (73.7%), del(13q) (82.2%), no aberrations (86.7%) or +12 (96.9%) (*P*=0.04). The other clinico-biological variables had no significant impact on the ORR (*Online Supplementary Table S2*).

The TTNT at 12 months was 18.1% (95%CI: 12.6-22.2) (median 31.3 months) (*Online Supplementary Figure S2*). A shorter TTNT was associated with del(17p) (median 20.2 months *vs.* 34.6) and with the group of patients who received previous CIT *vs.* chemotherapy as front-line regimen (27.2 months *vs.* 40.4) (Table 3). An U-IGHV status (29.3 months *vs.* 45.7) and the presence of 2 or more

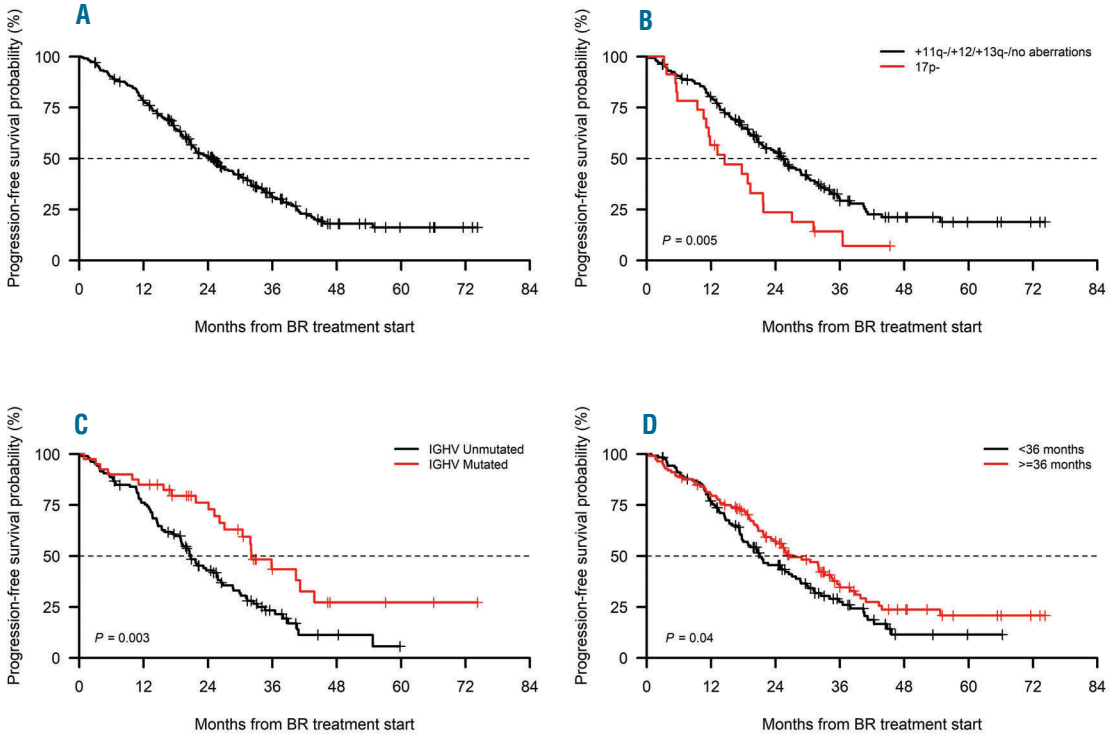

Figure 1. Progression-free survival (PFS) of patients treated with bendamustine and rituximab (BR) second-line. PFS of all 237 patients (A), by fluorescence *in situ* hybridization (B), IGHV status (C), and interval between first-line and second-line treatments (D).

comorbidities (27.2 months *vs.* 39.1) were of borderline significance, whereas age and the creatinine clearance had no impact on TTNT. First-line treatment with a CIT regimen was the only predictive factor for a shorter TTNT at multivariate analysis.

Seventy-three patients died due to CLL (n=14), infection with or without active CLL (n=27), second primary tumors (n=7), Richter's syndrome (n=4). In 12 patients, the cause of death was not reported. Other causes of death in single patients (n=9) are listed in *Online Supplementary Table S3*.

Overall survival at 12, 36 and 60 months was 92.7%, 72.2% and 54%, respectively, with a median OS of 74.5 months (Figure 2). Fifty-eight percent of patients in advanced stage were alive at 36 months compared to 75.8% in stage 0-II; 42.4% of patients who did not respond to BR were alive at 36 months compared to 78.2% of those who responded. An advanced stage (i.e. Rai stage III-IV or Binet stage C) and resistant disease were the only parameters significantly associated with a shorter OS at univariate and multivariate analysis (Table 4 and Figure 2).

Safety

A detailed report of grade 3-5 adverse events (AE) is shown in *Online Supplementary Table S4*. Thirty-three percent of patients (n=79) reported at least one grade 3-4 AE. Overall, cytopenia was recorded in 24.9% of patients. Grade 3-4 neutropenia (including febrile neutropenia) occurred in 20.7% of cases, thrombocytopenia in 6 patients (2.5%), anemia in 3 patients (1.2%), 2 of whom had autoimmune hemolytic anemia. Grade 3-5 infections were recorded in 16 patients including 4 with febrile neutropenia (6.7%), 8 of whom (3.4%) had a lung infection. One case of fatal infection was reported (encephalitis). Rash and/or dermatitis were reported in 2 patients (0.8%).

Efficacy of ibrutinib in the UK CLL forum and in the Italian Named Patient Program

Ninety-five patients were treated in 2014-2015 with single agent ibrutinib in second-line within the NPP (73 in the UK and 22 in Italy). Median follow up in the UK cohort was 3.1 years. These 95 patients were heterogeneous in baseline risk factors (Table 1), with an Eastern Cooperative Oncology Group (ECOG) PS≥2 being the

Table 2. Progression-free survival (PFS) with bendamustine and rituximab (BR) in second-line: univariate and multivariate analysis.

Variable	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age, years ≤65 *vs.* >65	0.899 (0.636-1.271)	0.5467	–	–
Sex, F *vs.* M	1.110 (0.787-1.566)	0.5519	–	–
Stage others *vs.* Rai III/IV or Binet C	0.676 (0.454-1.005)	0.0529	0.536 (0.319-0.903)	0.0192
Bulky lymph nodes (>5cm) yes *vs.* no	1.643 (0.959-2.815)	0.0705	–	–
Comorbidities 0-1 *vs.* ≥2	1.159 (0.844-1.592)	0.3625	–	–
Creatinine clearance (mL/min) ≤70 *vs.* > 70	1.179 (0.846-1.643)	0.3312	–	–
CD38 (>20%) neg *vs.* pos	0.841 (0.531-1.330)	0.4587	–	–
FISH 17p- *vs.* others	1.965 (1.214-3.180)	0.0060	2.92 (1.61-5.296)	0.0004
IGHV mutated *vs.* unmutated	0.484 (0.297-0.787)	0.0035	0.53 (0.299-)0.94	0.0299
Months between 1st and 2nd treatment < 36 *vs.* ≥36	1.398 (1.018-1.921)	0.0387	–	–
First-line chemo *vs.* CIT	0.846 (0.612-1.168)	0.3088	–	–
<6 cycles and/or dose reductions no *vs.* yes	0.752 (0.547-1.034)	0.0794	–	–

HR: Hazard Ratio; CI: Confidence Interval; Chemo: chemotherapy; CIT: chemoimmunotherapy; F: female; M: male.

Table 3. Time to next anti-leukemic treatment with bendamustine and rituximab second-line: univariate and multivariate analysis.

Variable	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age (years) ≤65 *vs.* >65	1.299 (0.918-1.838)	0.1400	–	–
Sex, F *vs.* M	1.040 (0.716-1.511)	0.8349	–	–
Stage others *vs.* Rai III/IV or Binet C	0.881 (0.557-1.393)	0.5873	–	–
Bulky lymph nodes (>5 cm) yes *vs.* no	1.598 (0.877-2.912)	0.1256	–	–
Comorbidities 0-1 *vs.* ≥2	1.372 (0.978-1.923)	0.0671	–	–
Creatinine clearance (mL/min) ≤ 70 *vs.* > 70	0.833 (0.579-1.199)	0.3261	–	-
CD38 (>20%) neg *vs.* pos	1.079 (0.650-1.793)	0.7678	–	–
FISH 17p- *vs.* others	1.863 (1.096-3.166)	0.0215	–	–
IGHV mutated *vs.* unmutated	0.597 (0.345-1.033)	0.0653	–	–
Months between 1st and 2nd treatment < 36 *vs.* ≥36	1.044 (0.741-1.469)	0.8062	–	–
First-line chemo *vs.* CIT	0.586 (0.407-0.843)	0.0040	0.59 (0.41-0.84)	0.0040
<6 cycles and/or dose reductions no *vs.* yes	0.776 (0.546-1.104)	0.1593	–	–

HR: Hazard Ratio; CI: Confidence Interval; Chemo: chemotherapy; CIT: chemoimmunotherapy; F: female; M: male.

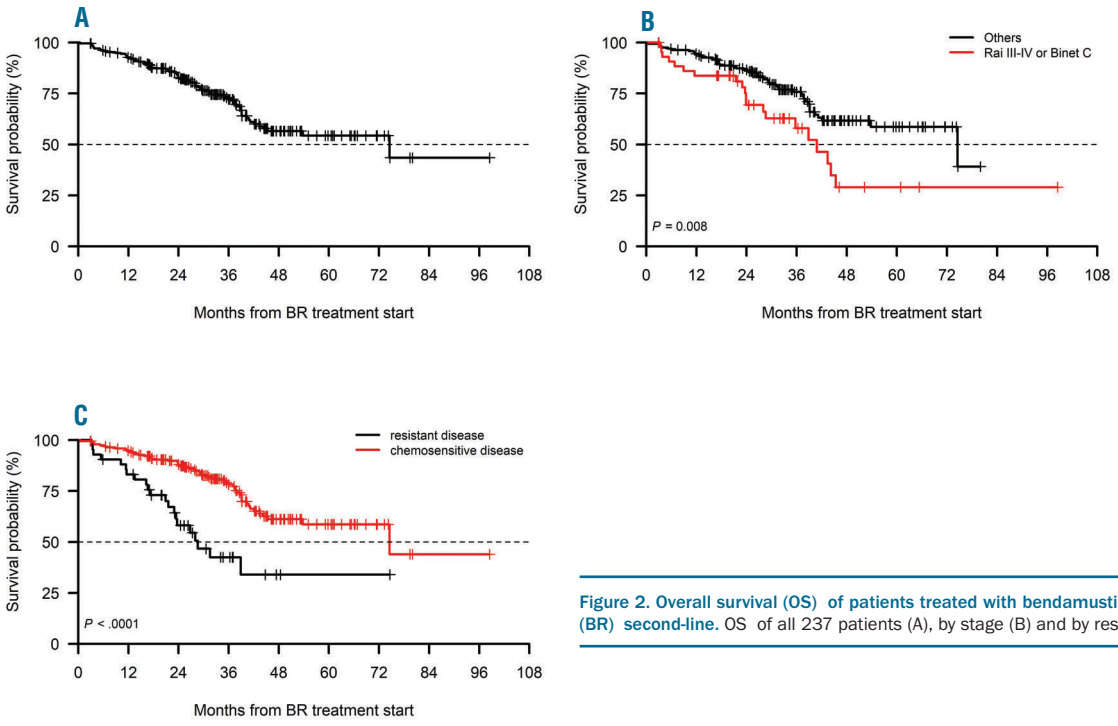

Figure 2. Overall survival (OS) of patients treated with bendamustine and rituximab (BR) second-line. OS of all 237 patients (A), by stage (B) and by response to BR (C).

only predictive factor with borderline statistical significance of shorter survival (*Online Supplementary Table S5*). When restricting the analysis to patients who had received CIT front-line (Table 5), the ibrutinib cohort and the BR cohort were comparable in terms of median age, ECOG PS, ORR rate to first-line treatment, and frequency of U-IGHV (available in a proportion of cases), although with a slightly shorter interval between first- and second-line treatment in the ibrutinib cohort (interval <36 months in 76.1% *vs.* 59.1% of patients) and a higher number of patients with 17p deletion in the ibrutinib cohort (36.1% *vs.* 14.8%). When excluding patients with del(17p) from the analysis, there was no significant difference in OS between the 39 patients treated with ibrutinib (63% alive at 36 months, 95%CI: 48.8-81.6) and the 92 patients treated with BR (74.4% alive at 36 months, 95%CI: 64.7-85.5 (Figure 3). A subanalysis of the OS in patients with intact 17p and with a less than 36-month interval between first-line and first salvage treatment in the BR cohort (n=55) and in the ibrutinib cohort (n=33) showed no significant difference, with 72.6% of patients alive at three years with BR (95%CI: 60.1-87.7) and 59.8% alive at three years with ibrutinib (95%CI: 44.2-80.7) (*P*=0.19).

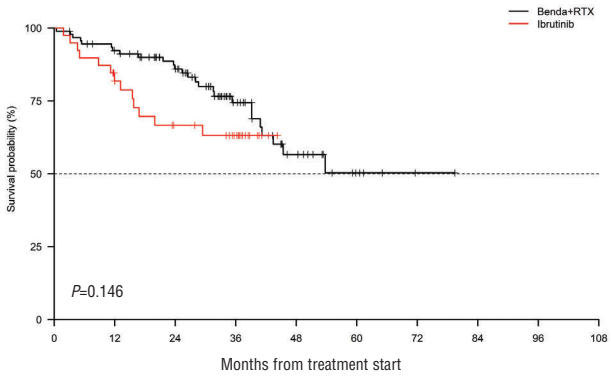

Figure 3. Indirect comparison of overall survival in 39 patients treated second-line with ibrutinib and in 92 patients treated with bendamustine and rituximab (Benda+RTX). All patients had intact 17p and received chemoimmunotherapy as front-line therapy.

Discussion

Accepting the limitations of retrospective analyses, we set out to collect data on the efficacy of BR, one of the most widely utilized CIT regimens in CLL. We elected to include in this study only patients who received second-line treatment with BR given the limited availability of published data in this setting in order to contribute new information that may assist clinicians in the selection of the most appropriate first salvage treatment in CLL. To minimize possible selection biases and imprecise reporting of data: i) we encouraged clinicians to report all patients who initiated BR treatment; ii) we analyzed the reported data according to the intention-to-treat principle; and iii) we performed computerized and manual consistency checks on each case report form.

Besides PFS, we included objective efficacy measures of the BR regimen, such as OS and the TTNT. Keeping in mind that response assessment may vary among centers and that bone biopsy was not routinely performed, we agreed to record as "response" what each treating clinician graded as "partial" or "complete" remission.

The patient population who received BR included in this study closely resembled the typical CLL patient seen in daily clinical practice in terms of age, PS and comorbidities.[14] The number of patients who completed the planned treatment (69.6%) was in line with a previous prospective phase-II GIMEMA study, where 76% of R/R CLL patients completed treatment.[16] This finding suggests that there

Table 4. Overall survival after univariate and multivariate analysis.

	Univariate HR (95% CI)	P	Multivariate HR (95% CI)	P
Age, years ≤65 *vs.* >65	0.741 (0.439-1.250)	0.2612	–	–
Sex, M *vs.* F	0.836 (0.491-1.425)	0.5107	–	–
Stage, others *vs.* Rai III/IV or Binet C	0.501 (0.296-0.846)	0.0098	0.547 (0.320-0.935)	0.0276
Bulky lymph nodes (>5 cm) yes *vs.* no	1.161 (0.464-2.905)	0.7492	–	–
Comorbidities 0-1 *vs.* ≥2	1.069 (0.671-1.702)	0.7797	–	–
Creatinine clearance (mL/min) ≤70 *vs.* >70	1.401 (0.850-2.308)	0.1855	–	–
CD38 (>20%) neg *vs.* pos	0.722 (0.356-1.465)	0.3665	–	–
FISH 17p- *vs.* others	1.500 (0.734-3.064)	0.2663	–	–
IGHV mutated *vs.* unmutated	0.604 (0.290-1.254)	0.1761	–	–
Months between 1st and 2nd treatment <36 *vs.* ≥36	1.496 (0.934-2.398)	0.0941	–	–
First-line chemo *vs.* CIT	0.977 (0.609-1.565)	0.9216	–	–
<6 cycles and/or dose reductions no *vs.* yes	0.706 (0.444-1.123)	0.1419	–	–
ORR CR; Cri; PR; nPR/*vs.* PD; SD; NR	0.330 8 (0.197-0.552)	<.0001	0.344 (0.198-0.595)	0.0001

HR: Hazard Ratios; CI: Confidence Interval; Chemo: chemotherapy; CIT: chemoimmunotherapy; ORR: overall response rate; CR: complete remission with incomplete marrow recovery; nPR: nodular partial response; F: female; M: male; NR: no remission; PD: progressive disease; PR: partial remission; SD: stable disease.

Table 5. Baseline characteristics of patients treated with chemoimmunotherapy in first-line in the bendamustine and rituximab (BR) and in the ibrutinib cohorts (UK + NPP GIMEMA).

Variable	BR (n=137)	Ibrutinib (n=71)	P
Median age, years (range)	68.2 (39.4-84.6)	67.1 (27.5-85.3)	0.603
Age, years (%) ≤65/>65	39 (34.5)/74 (65.5)	27 (38.6)/43 (61.4)	0.691
Sex, (%) M/F	91 (66.4)/46 (33.6)	45 (63.4)/ 26 (36.6)	0.777
ECOG PS (%) 0-1/≥2	113 (91.9)/10 (8.1)	57 (82.6)/ 12 (17.4)	0.090
Months between 1st line and 2nd line			
Median (range)	30.60 (0.40, 79.40)	19.40 (1.80, 77.60)	0.001
n. <36/≥36 (%)	81 (59.1)/56 (40.9)	54 (76.1)/17 (23.9)	0.023
Response to 1st line treatment (%) no/yes	28 (20.4)/109 (79.6)	8 (15.1)/45 (84.9)	0.524
IGHV (%) mutated/unmutated	17 (19.5)/70 (80.5)	8 (32.0)/17 (68.0)	0.295
17p- (%) yes/no	16 (14.8)/92 (85.2)	22 (36.1)/39 (63.9)	0.003

NPP GIMEMA: Named Patient Program-Gruppo Italiano Malattie Ematologiche dell'Adulto; ECOG: Eastern Cooperative Oncology Group; n: number. F: female; M: male; PS: performance status.

was minimal, if any, patient selection bias in our study. The number of grade 3-4 infections (6.7%) is similar to the 4.2% incidence of severe infections in a trial using bendamustine and ofatumumab in patients who had received 1-2 previous lines of treatment.[16] In another trial using the BR regimen, the incidence of grade 3 infections was 12.8% in patients who had received 1-5 previous lines of treatment.[15] Thus, our data show that BR is a relatively safe second-line regimen in terms of infectious complications in a real-life population. The lower incidence of grade 3-4 cytopenias in this study compared to other prospective studies showing a 50-78% incidence of grade 3-4 cytopenias[15,16] reflects the policy not to perform a blood count in the routine practice at the nadir time point at many centers.

With a 78.6% PFS rate at 12 months (median 25 months), a 31.3-month median TTNT, and a 92.7% OS rate at 12 months (median 74.5 months), our data show that the BR regimen is an effective first salvage regimen. Interestingly, the efficacy of this regimen in terms of PFS, TTNT and OS was not influenced significantly by age, creatinine clearance, by the presence of 2 or more comorbidities. PFS was negatively influenced by advanced stage, del(17p) and U-*IGHV*, confirming the strong prognostic significance of these parameters[24] also in the second-line setting. Patients without any of these unfavorable characteristics experienced a prolonged median PFS (40.4 months).

Although PFS estimation should be interpreted with caution in a retrospective analysis, our data are similar to those observed in a prospective phase II GIMEMA trial[16] that reported a median PFS of 23.6 months with bendamustine and ofatumumab in 49 R/R CLL (61% with 1 previous treatment, 39% with 2 previous lines). In another analysis of BR in patient who had received a median number of 2 previous treatments (range 1-5), the median PFS was 15.2 months (95%CI: 12.5-17.9 months).[15] A 18-month median PFS was reported with BR as first salvage after fludarabine, cyclophosphamide and rituximab in 62 patients.[13]

Time to next treatment was longer in those patients who had received chemotherapy as first-line treatment. It is worth noting that even though published guidelines proposed the preferential usage of CIT, 27.9% of our patients who started treatment after 2010 had received only chemotherapy as initial treatment.

The survival data in our analysis (92.7% alive at 12 months and 72.2% at 36 months) reflect previous experiences with bendamustine and anti-CD20 in clinical trials,[16,25] showing that this combination is equally effective in clinical practice across many centers. Negative predictive factors on OS were represented by advanced stage and chemorefractory disease, whereas the presence of del(17p) was not associated in our analysis with a significantly shorter OS, possibly due to the relatively low number of patients (n=23) and to the use of effective salvage regimens in subsequent lines of treatment. Accordingly, the survival in the BR arm of the Helios trial after adjusting for crossover to BR and ibrutinib was close to 90% at 12 months and more than 80% at 24 months.[26]

Thus, the data presented here show that BR is an efficacious first salvage regimen in CLL in a real-life population, including elderly patients, patients with 2 or more comorbidities and a creatinine clearance less than 70 mL/min. The outcome was better in patients with favorable genetic features and with early/intermediate disease stage. Importantly, no significant differences in terms of OS were noted in this real-life report with respect to the survival data recently observed in clinical trials.

Because no direct comparison was performed between CIT and new oral agents in first relapse, we elected to compare our data with ibrutinib used in a real-life patient population treated in the UK and in Italy using OS as an objective end point. We restricted our comparative analysis to patients who had previously received CIT because this is the recommended initial treatment in CLL. The BR and ibrutinib cohorts had similar baseline risk factors and, when excluding patients with del(17p) from the analysis as nowadays they would no longer be exposed to second-line CIT, there was no difference in OS (Figure 3). Interestingly, no difference in OS was found with BR or ibrutinib when including in the subanalysis patients with a less than 36-month interval between front-line and first salvage treatment. The survival curve showed an excess of early deaths in the ibrutinib cohort compared to the BR cohort. Due to the small size of the patient population included in this analysis, there is no recurrent pattern or obvious explanation for this observation; severe infection and Richter's syndrome in 2 patients each were the only recurrent causes of death in the first 12 months. It remains unclear as to whether ibrutinib directly contributed to any of these deaths.

The observed outcome for ibrutinib-treated patients in this observational study could be due to premature interruption of ibrutinib exposure. It is noteworthy that in the UK and Ireland data on the overall cohort of R/R CLL treated with ibrutinib, discontinuations during the first year were due to AEs (54%), Richter's transformation (26%), and progressive CLL (17%). Beyond the first year, the rate of discontinuations due to progressive CLL increased to 29%.[27]

When comparing CIT and novel inhibitors, one also has to consider the long-term detrimental effects due to the clonal selection and DNA damage occurring with repetitive lines of chemotherapy-based treatments, resulting in second tumors, acute leukemias/myelodysplastic syndromes, that cannot be evaluated in the short follow up of our retrospective study. On the other hand, elegant *in vitro* studies have shown that treatment of mouse B cells with idelalisib or duvelisib, and to a lesser extent ibrutinib, increased somatic hypermutation through enhanced expression of activation-induced cytidine deaminase.[28]

In another analysis including R/R CLL in second and subsequent lines of treatment,[29] an OS advantage was noted when comparing ibrutinib (with or without BR) and BR alone.[30] In other studies arriving at the same conclusion,[31,32] chemotherapy +/- anti CD20 regimens used in a real-world setting were compared with ibrutinib data of the clinical trials Resonate and Helios. However, there is now evidence that adherence to treatment with ibrutinib in the real-world population did not reflect the data obtained in clinical trials,[22,33] possibly due to the heterogeneity of the patient populations or to a more limited experience of physicians in managing side effects occurring on treatment. Furthermore, the UK real-life data show that duration of ibrutinib therapy and OS seem very similar when ibrutinib is used at first or subsequent relapses, suggesting that the relative benefit for ibrutinib compared with chemotherapy is more evident in patients with multiple relapse where re-treatment with further chemotherapy results in progressively worse response rates and remission duration. It is noteworthy that a highly significant PFS advantage with the BCL2 inhibitor venetoclax plus rituximab compared to BR has been recently reported in the planned interim analysis of the randomized phase III Murano study, where 42.8% of patients had received 2 or more lines of therapy.[34] However, in this trial, an OS benefit has not yet been shown according to the predefined statistical model.

Although, obviously, data derived from different series must be treated with caution, these data suggest that BR and ibrutinib may be equally effective in terms of OS when used as first salvage treatment in CLL patients without 17p deletion managed in the real-life setting. Whether this is due to limited compliance of patients and/or suboptimal management of side effects with the novel therapies remains to be established.

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