Received: 15 September 2024 | Accepted: 13 November 2024

DOI: 10.1111/bjh.19920

BJHaem
BRITISH JOURNAL OF HAEMATOLOGY

ORIGINAL PAPER

Haematological Malignancy – Clinical

Efficacy and safety of bendamustine, rituximab and bortezomib treatment in relapsed/refractory Waldenstrom Macroglobulinaemia: results of phase 2 single-arm FIL-BRB trial

Giulia Benevolo[1] | Daniela Drandi[2] | Nicoletta Villivà[3] | Anna Castiglione[4] | Federico Monaco[5] | Carola Boccomini[6] | Daniela Dessi[7] | Catello Califano[8] | Luigi Curreli[9] | Federica Cavallo[1,2] | Annarita Conconi[10] | Gianluca Gaidano[11] | Francesca Gaia Rossi[12] | Tommaso Caravita di Toritto[3] | Martina Ferrante[2] | Donato Mannina[13] | Patrizia Tosi[14] | Giuseppe Pietrantuono[15] | Gerardo Musuraca[16] | Michele Merli[17] | Roberto Sartori[18] | Monica Tani[19] | Roberto Freilone[6] | Marzia Varettoni[20] | Simone Ferrero[1,2]

Correspondence
Giulia Benevolo, AOU Città della Salute e della Scienza di Torino—Ematologia U, Turin, Italy.
Email: gbenevolo@cittadellasalute.to.it

Summary

This multicentre phase II study Fondazione Italiana Linfomi (FIL)-bortezomib plus rituximab plus bendamustine (BRB) tested a combination of bendamustine (90 mg/m^2 on days 1–2), rituximab (375 mg/m^2 intravenously on day 1) and bortezomib (1.3 mg/m^2 sc on days 1, 8, 15, 22) every 28 days for six cycles in 38 symptomatic patients with relapsed/refractory Waldenstrom macroglobulinaemia (RR-WM). Moreover, MYD88^{L265P} and CXCR4^{S338X} mutations were tested by droplet digital polymerase chain reaction (ddPCR) both at baseline and at the end of treatment in 21 patients. Overall response rate at the end of therapy was 84.6%, including 4 (11%) complete remission, 15 (39%) very good partial response, 12 (32%) partial responses according to IWWM response criteria. At 18, 24 and 30 months, progression-free survival was 84.2% (95% CI 68.2%–92.6%), 81.5% (95%CI 65.1–90.7) and 78.8% (95%CI 62.0–88.8) respectively. At 18 months, the Overall survival was 92.1% (95%CI 77.5%–97.4%). Overall, 19 patients (50%) experienced grade 3–4 haematological toxicity, mainly thrombocytopenia, and grade 1–3 neuropathy rate was about 10% and required bortezomib dose reduction but did not result in treatment interruption. Moreover, BRB treatment induced the high rates of undetectable molecular minimal residual disease (MRD) at the end of the therapy. BRB regimen used as second line is an effective and well-tolerated salvage treatment for relapsed refractory Waldenstrom macroglobulinaemia patients. MRD monitoring showed promising efficacy in clearing the residual disease.

KEYWORDS

chemotherapy, immunotherapy, molecular biology, Waldenstrom-S macroglobulinaemia

For affiliations refer to page 8.

INTRODUCTION

Waldenström's macroglobulinaemia (WM) is a low-grade B-cell lymphoplasmacytic lymphoma, characterized by the presence of a monoclonal immunoglobulin-M (IgM) protein, lymphoplasmacytic bone marrow (BM) infiltration, cytopenia, hepatomegaly, splenomegaly and lymphadenopathy.[1,2]

In 2024, the treatment scenario includes a wide range of approaches including monoclonal antibodies, alkylating agents, proteasome inhibitors and Bruton tyrosine kinase inhibitors (BTKi).[3] However, despite several treatment options, WM patients invariably experience relapse, with a median survival of 10–12 years from the time of diagnosis.[3]

Symptomatic patients with relapsed/refractory Waldenstrom macroglobulinaemia (RR-WM) treated with standard rituximab plus chemotherapy as second-line salvage therapy generally show an 18-month progression-free survival (PFS) of about 50%.[4,5] Encouraging results have been obtained with combination of rituximab plus bendamustine[6] or rituximab plus bortezomib[7] with an acceptable toxicity, mainly neutropenia for bendamustine and neuropathy for bortezomib.

More recently, the BTKi approach has become standard therapy at relapse (18-month PFS rate about 75%–80%), however, with a no negligible number of adverse events (AEs) including atrial fibrillation, hypertension, bleeding, cytopenia and infections.[3]

The aim of this trial, sponsored by the *Fondazione Italiana Linfomi* (*FIL*), was to determine the efficacy and safety of bortezomib plus rituximab plus bendamustine (BRB) in order to offer a further therapeutic opportunity in patients with RR-WM.

METHODS

Study design

This is a multicentre phase 2 study evaluating a combination of BRB in WM at first relapse; in a second time (as of 15 September 2015), a biological amendment was implemented to include a molecular study evaluating *MYD88* and *CXCR4* mutational status of patients and to assess minimal residual disease (MRD) after treatment. Here, we present the final clinical and molecular results of the FIL-BRB study. The study was conducted according to the International Conference on Harmonization Guideline for Good Clinical Practice and to the Declaration of Helsinki. The protocol and its appendices were subject to review and approval by the competent Independent Ethics Committee(s). All patients provided written consent. This trial was registered at http://www.clinicalstrial.gov(NCT02371148) (EudraCT Number: 2013-005129-22).

Patients

Enrolment lasted from October 2014 until November 2017. Data updates were performed in June 2021 and final analysis was performed in June 2023 (the delay was due to the SARS-CoV-2 (COVID-19) pandemic which slowed down all clinical and research activity).

Eligible participants were adults older than 18 years who had a clinicopathologic diagnosis of lymphoplasmacytic lymphoma/WM according to the REAL/WHO classification and had relapsed/refractory disease after receiving first-line chemotherapy. If patients previously received bortezomib or bendamustine, they must had obtained a partial response lasting at least 2 years. Active disease was defined by the presence of at least one of the following criteria: constitutional symptoms, haemoglobin less than 10 g/L, platelets less than 100×10^9/L, symptomatic splenomegaly, bulky disease (>7 cm), hyperviscosity syndrome, IgM-related peripheral neuropathy up to grade 1, haemolytic anaemia or immune complex vasculitis.

Intervention

Treatment protocol consisted of six 28-day cycles with: bortezomib 1.3 mg/sqm sc days 1, 8, 15, 22; rituximab: 375 mg/sqm iv day 1 (at cycle 1, rituximab could be given on day 8 in order to reduce the risk of paradoxycal IgM flare); bendamustine 90 mg/sqm iv days 1–2 or days 2–3 according to institutional/physician choice.

Janssen Pharmaceuticals, the manufacturer of bortezomib, provided drug free of charge and funds to carry on the research. Rituximab and bendamustine were obtained commercially.

The treatment schedule is displayed in Figure 1.

Prophylactic use of valacyclovir 500 mg once a day or acyclovir 400 mg twice a day and trimethoprim/sulphamethoxazole (180/800 mg) 1 cp/day × 3 days/weeks was recommended up to 6 months after the last anti-CD20. Additional prophylaxis with levofloxacine or ciprofloxacine, fluconazole or itraconazole and granulocyte colony-stimulating factor (G-CSF) was administrated in case of neutropenia $<1.0 \times 10^9$/L.

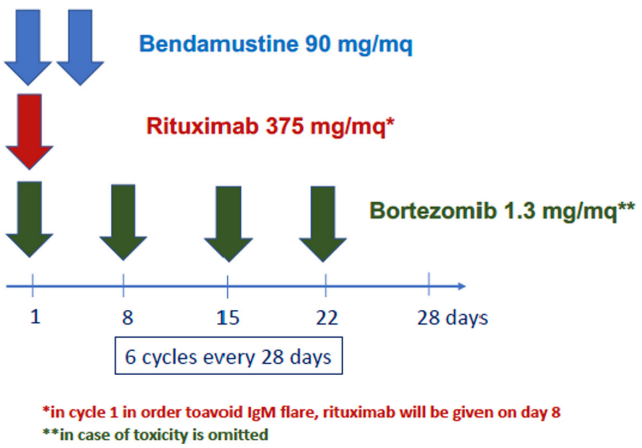

FIGURE 1 Treatment schedule.

Outcomes

Duration of treatment was 6 months (one cycle per month) plus 60 days for response evaluation.

Primary outcome of FIL-BRB was PFS, defined as time from the treatment beginning to the date of disease progression, relapse or death from any cause. Patients without any relapse at the end of the follow-up were censored at their last assessment date. Minimum follow-up time required for all patients was 2 years.

Secondary outcomes were overall response rate (ORR), overall survival (OS) and toxicity.

Response criteria were defined according to WM consensus recommendations of the 6th International Workshop on WM.[8]

OS was defined as time from the treatment beginning to the date of death from any cause. Patients alive at the time of the final analysis were censored at the date of the last contact.

Toxicity was defined according to "Common Terminology Criteria for Adverse Events" (CTCAE), version 4.0 (http://ctep.cancer.gov/reporting/ctc.html).

To monitoring neurological toxicities at each cycle, patients had to complete a neuropathy questionnaire (FACT/GOG-Neurotoxicity Questionnaire, Version 4.0).

BIOLOGICAL STUDY (FIL-BIO-BRB)

Sample requirements

Biological samples were collected on site ad sent to the reference molecular laboratory (Division of Hematology, Torino University, Italy) both at baseline and at the end of therapy. For each time point, 7 mL of bone marrow (BM) aspirate in sodium/citrate tubes and 20 mL of peripheral blood (PB) were collected in Streck BCT tubes.

Genomic DNA (gDNA) was extracted from white blood cells (WBC) by MaxWell RSC system with blood RSC kit (Promega), while cfDNA was extracted by Maxwell RSC with LV ccfDNA kit (Promega), in accordance with the manufacturer recommendations.

Methods

MYD88^{L265P} was tested by droplet digital polymerase chain reaction (dd-PCR) in BM, PB and cfDNA samples, at baseline and at the end of treatment, as previously reported.[9]

CXCR4 mutations were tested by a drop-off droplet digital polymerase chain reaction (ddPCR) assay for *CXCR4*^{S338X} (p.S388) mutation (*CXCR4*^{MUT}) detection in BM samples at baseline and at end of treatment, with a sensitivity of 0.001%.[10] Finally, in order to cover also the CXCR4 mutations outside the most frequent p.S388 locus, targeted next-generation sequencing (NGS) targeted resequencing was performed in the same BM samples by Illumina Hiseq 2500 with a median coverage of 2369x in the laboratory of Pavia, Italy.[11]

Statistical analysis

This phase II trial was conducted according to a single-arm design with a primary time-to-event end-point, based on a non-parametric survival distribution. The interactive One Arm Nonparametric Survival Sample Size and Power program (provided by SWOG) was used to calculate the sample size.

According to literature, the following two hypotheses were considered: a PFS lower than 50% at 18 months is of no further interest; and second, a PFS of 65% was clinically meaningful. With an alpha error of 0.10 (one-sided), a beta error of 0.20, 24 months of follow-up from the last enrolled patient, the required sample size was of 38 patients. An intention-to-treat analysis was conducted, assuming that there were no patients lost to follow up. Time-to-event variables were analysed using the Kaplan–Meier method. Descriptive statistics and their 95% confidence intervals were used to summarize the activity and the safety end-points measured as proportions.

RESULTS

Patients and disease characteristics

From October 2014 to November 2017, a total of 38 consecutive eligible patients were enrolled in 18 FIL centres. Baseline characteristics of patients are listed in Table 1. Most of the patients were male; median age was 67 years and eight patients were older than 75. Forty-two per cent of patients had at least one comorbidity, mostly cardiovascular disease (21%) or metabolic disorders (16%), such as diabetes. At baseline, we observed elevated IgM values (median value 42 g/L, range 1.37–99.80) and low haemoglobin level (median value 9.3 g/L, range 7.4–13.2 g/L). Moreover, constitutional symptoms (39%) and symptomatic splenomegaly (24%) were frequent. The revised ISSWM[12] was intermediate (21%), high (18%) or very high (16%) in about half of patients.

Median time from diagnosis and from first therapy was 62.4 (interquartile range 40.8; 90.6) and 40.8 (interquartile range 28.3; 61.3) months respectively. First-line therapies were mainly CRD (21 patients; 55%) and RCVP (7 patients, 18.4%), one patient previously received bortezomib therapy in the RBD schedule while no patient was previously treated with bendamustine nor with BTK-i. Three patients received BRB within 180 days of first-line therapy (refractory patients). The study flowchart is reported in Figure 2. Among the 38 enrolled patients, six patient discontinued treatment due to adverse events and two patients for progression disease. For this reason, 30 of 38 (79%) patients completed all planned cycles of therapy.

TABLE 1 Characteristics at baseline of enrolled patients ($n = 38$).

	No.	%
Sex		
Female	10	26.3
Male	28	73.7
Age		
≤65	18	47.4
66–75	12	31.6
>75	8	21.1
Symptomatic disease		
Presence of systemic symptoms	15	39.5
Haemoglobin < 10 g/dL	27	71.1
Platelets < $100 \times 10^{9/L}$	6	15.8
Presence of symptomatic splenomegaly or bulky disease	9	23.7
Presence of hyperviscosity syndrome	11	28.9
Presence of peripheral neuropathy grade = 1	4	10.5
Presence of haemolytic anaemia (warm AIHA)	3	7.9
Presence of immune complex vasculitis	3	7.9
Comorbidity		
At least one	16	42.1
Cardiovascular disease	8	21.1
Metabolic disease	6	15.8
Pulmonary disease	2	5.3
Neurological disease	2	5.3
Neoplastic disease	1	2.6
Other disease	11	28.9
rISSWM[12]		
Very low/low	17	44.7
Intermediate	8	21.1
High/	7	18.4
Very High	6	15.8
Time from diagnosis (months)—[median; interquartile range]	5.0	0.9–47

Outcomes

Primary end-point

At 18, 24 and 30 months, PFS was 84.2% (95% CI 68.2%–92.6%), 81.5% (95%CI 65.1–90.7) and 78.8% (95%CI 62.0–88.8) respectively (Figure 3). During follow-up, we observed 15 PFS events (12 progressions/relapses and five deaths, two of them after relapse and/or progression). Three patients experienced disease progression after 36 months from the beginning of therapy. The median follow-up time was 39.26 months (95%CI 36.37–43.46).

Secondary end-point

The ORR was 84.6% (95% CI 65.7; 92.3%), including 4 (10.5%) complete remission (CR), 15 (39.5%) very good partial remission (VGPR), 12 (31.6%) partial remission (PR), 1 (3%) minimal response (MR) and 1 (3%) stable disease (SD), according to IWWM response criteria.

At 18 months, the OS was 92.1% (95%CI 77.5%–97.4%) and no deaths were observed between 18 and 36 months (Figure 4). During follow-up, we observed five deaths (2 due to infections, 1 due to metastatic lung cancer, 1 due to cerebrovascular accident during the fifth cycle and 1 due to pulmonary embolism during the third month of follow-up) of which two occurred after 36 months from the beginning of therapy, at 44 and 54 months respectively.

Toxicity

Nineteen patients (50%) experienced grade 3–4 haematological toxicity, mainly neutropenia (14 pts) or thrombocytopenia (5 pts). All patients with grade 3–4

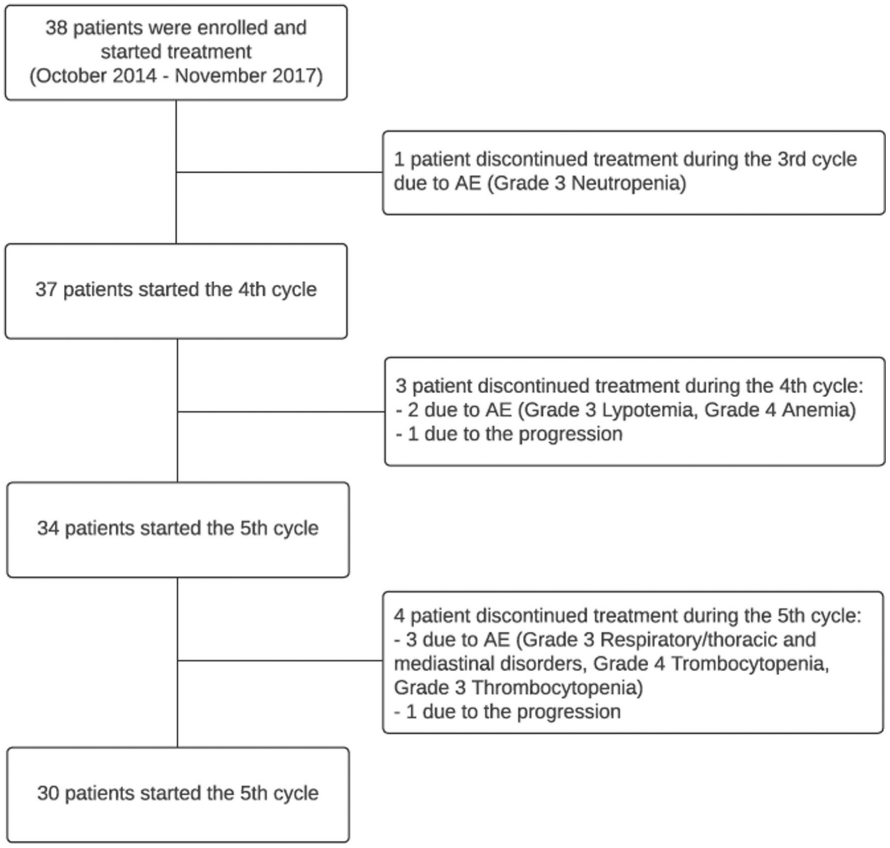

FIGURE 2 Study flowchart.

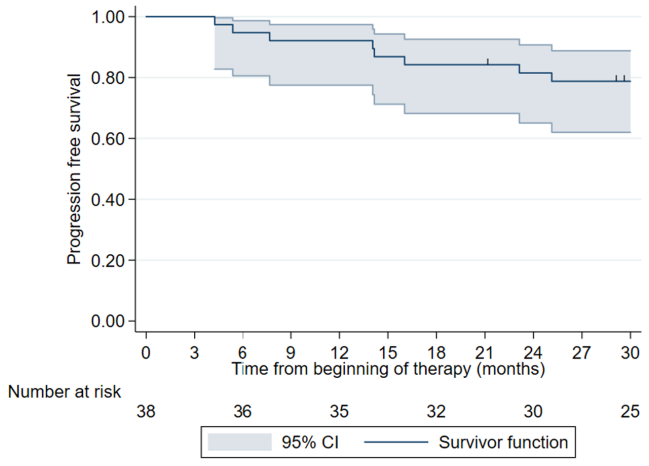

FIGURE 3 Progression-free survival.

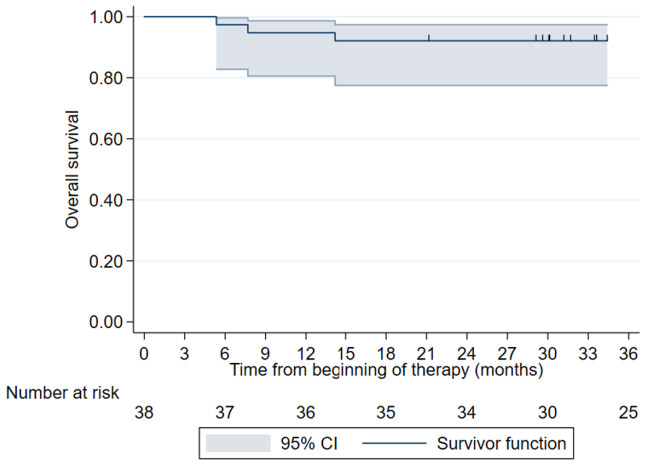

FIGURE 4 Overall survival.

thrombocytopenia interrupted treatment due to this toxicity; instead, no patient discontinued due to neutropenia and the incidence of infections was low, probably due to mandatory prophylaxis (infections rate <10%, mainly grade 1–2). Twelve patients (31.6%) developed grade 3–4 extra-haematological toxicity of which only one cutaneous toxicity related to bendamustine. Instead, grade 1–2 toxicity was mainly extra-haematological with gastrointestinal disorder or neuropathy (neuropathy rate about 10%) and required bortezomib dose reduction in four patients but did not result in treatment interruption. Interestingly, among the four patients who presented with neuropathy at the start of therapy, none developed clinical worsening, valuated by neuropathy questionnaire, during treatment with bortezomib. Moreover, the only patient who had previously been treated with bortezomib did not present neuropathy at the screening and did not develop neurological toxicity during therapy. Toxicities are listed in Table 2.

Molecular analysis

Mutational data were available for 21 patients: All patients scored $MYD88^{L265P}$ mutated in BM, 18/19 (95%) in cfDNA and only 18/21 (86%) in PB ($p = 0.220$; Table 3). Of note, the median mutational level of PB samples was more than 1 log lower compared to BM while $MYD88^{L265P}$ levels across cfDNA and BM were superimposable, with a median $MYD88^{L265P}$ quantification of 7, 1E-02 (range: 1E-03-8, 7E-01) in BM, 3, 8E-02 (range: 1, 4E-03-9, 4E-01) in cfDNA and 8, 9E-04 (range: 3, 6E-04-8, 7E-01) in PB.

In those patients with paired samples at diagnosis and post therapy, MRD negativity rates after treatment differed across the investigated tissues: In detail, 5/17 (29%) patient were negative in BM, 6/14 (43%) in plasma and 12/16 (75%) in PB ($p = 0.028$). The quantitative evaluation of MRD-positive cases showed a decrease of about 1 log, compared to baseline. In detail, 12/17 (71%) BM-positive samples showed a median of 3, 9E-03 (range 3, 5E-04–1, 3E-02) and 8/14 (57%) positive cfDNA a median of 4, 7E-03 (range: 4, 2E-04-1, 80E-02). The only 4/16 (25%) PB samples positive after therapy showed a median of 5, 5E-04 (range: 3, 7E-04–4, 6E-03).

CXCR4 mutation at locus p.S338 was detected by ddPCR in BM of one patient (1/21) both at baseline (with an AF of 22%) and after treatment (with an AF of 0.5%).

Furthermore, ddPCR results were compared with NGS in 21 BM samples at baseline showing a level of agreement of 71% between the two techniques. Fifteen patients were concordantly $MYD88^{L265P}$, while six cases ddPCR-MUT/NGS-WT were characterized by low mutational levels by ddPCR (median 2E-03).

CXCR4 mutations were detected by NGS in two BM samples: one concordant with ddPCR, while the second ddPCR WT case was characterized by a *CXCR4* mutation outside the p. S338X locus (p. E343fs).

TABLE 2 Haematological and extra-haematological toxicity.

	Any grade toxicity			Severe toxicity (grade ≥3)		
	***N* tox**	***N* pat**	**%**	***N* tox**	***N* pat**	**%**
Anaemia	5	5	13.16	2	2	5.26
Leucopenia	9	3	7.89	6	1	2.63
Neutropenia	69	19	50	54	17	44.74
Thrombocytopenia	27	8	21.05	13	5	13.16
Febrile neutropenia	0	0	0	0	0	0
Any haematological toxicity	**110**	**23**	**60.53**	**75**	**19**	**50.00**
Cardiac disorders	0	0	0	0	0	0
Congenital/familial/genetic disorders	0	0	0	0	0	0
Ear and labyrinth disorders	3	2	5.26	0	0	0.00
Eye disorders	1	1	2.63	0	0	0.00
Endocrine disorders	0	0	0	0	0	0
Gastrointestinal disorders	18	13	34.21	2	2	5.26
General disorders and administration site conditions	24	11	28.95	0	0	0.00
Hepatobiliary disorders	5	1	2.63	0	0	0.00
Immune system disorders	4	3	7.89	0	0	0.00
Infections and infestations	6	5	13.16	1	1	2.63
Metabolism and nutrition disorders	3	2	5.26	0	0	0.00
Musculoskeletal and connective tissue disorders	2	2	5.26	0	0	0.00
Neoplasms benign/malignant/unspecified (incl. cysts and polyps)	1	1	2.63	1	1	2.63
Nervous system disorders	10	6	15.79	2	1	2.63
Renal and urinary disorders	5	3	7.89	1	1	2.63
Reproductive system and breast disorders	3	3	7.89	1	1	2.63
Respiratory/thoracic and mediastinal disorders	10	9	23.68	5	5	13.16
Skin and subcutaneous tissue disorders	9	6	15.79	2	2	5.26
Surgical and medical procedures	4	1	2.63	2	1	2.63
Vascular disorders	1	1	2.63	1	1	2.63
Other (specify)	10	7	18.42	1	1	2.63
Any extra-haematological toxicity	**119**	**29**	**18.42**	**19**	**12**	**31.58**

Note: Bold values are descriptive data that has no statistical significance.

TABLE 3 Mutational status at baseline and at the end of therapy ($n = 21$).

		Baseline			End of therapy		
			Mutations			Mutations	
		N of samples	*N*	%	*N* of samples	*N*	%
MYD88	Bone marrow aspirate	19	19	100	17	12	71
	Peripheral blood	21	18	86	16	4	25
	Plasma	19	18	95	14	8	57
CXCR4	Bone marrow aspirate	19	1	5	7	1	14

DISCUSSION

In 2024, the treatment approaches in patients with RR-WM patients include a wide range of approaches. As recommended by consensus panel 2 (CP2) of the 11th International Workshop on WM (IWWM-11),[3] chemoimmunotherapy (CIT) and/or a BTKi strategies are important options and they must be selected based on biological age, co-morbidities, fitness, previous therapy, nature of relapse, disease phenotype and WM or therapy-related complications, patient preferences and haematopoietic reserve. Moreover, mutational status (*MYD88, CXCR4*) may influence the efficacy of some specific therapies (i.e. cBTKi) and should be evaluated in RR-WM patients.

CIT such as dexamethasone, rituximab and cyclophosphamide (DRC); bendamustine plus rituximab (BR); or bortezomib dexamethasone and rituximab (BDR); or BTK-i is valuable options for primary therapy in patients with symptomatic WM and can also be used in the management of RR disease. Nonetheless, there is no consensus on which treatment regimen provides the best safety and efficacy profile due to the lack of prospective randomized studies comparing these regimens. Importantly, there is no specific recommendation for fixed duration regimens (CIT) versus indefinite duration regimens (BTK-i). For patients with remissions lasting less than 12 months or who show progressive disease/resistance to a first-line regimen, second-line treatment must be switched to different drugs.

In this multicentre prospective phase II study, we investigated the efficacy and safety of the BRB regimen in patients with RR-WM. The current study is the first report on the use of combination of BRB in these patients.

Bortezomib has been shown to have high activity in the management of RR-WM, with response rates ranging from 81% to 96%.[3,7,13] In our study, in a setting of RR patients, we observed an ORR of 85%, with almost 50% of patients obtaining at least VGPR. These results were similar to those obtained in untreated patients with combination of bortezomib, rituximab and dexamethasone (BDR) (ORR of 85%)[14] or in RR-WM patients treated with BTKi (zanubrutinib; ORR 88%).[15]

Recently, Buske et al.[16] enrolled 202 patients to randomly receive DRC versus bortezomib-DRC (B-DRC). After a median follow-up of 27.5 months, the estimated 24-month PFS was 80.6% for B-DRC and 72.8% for DRC ($p = 0.32$). At the end of treatment, B-DRC and DRC induced major responses in 80.6% versus 69.9% and a CR/VGPR in 17.2% versus 9.6% of patients respectively. These data are similar with those obtained with BRB.

In addition, De Tute et al.[17] had recently demonstrated that combination with cyclophosphamide, rituximab and bortezomib (BRC) is a tolerable and highly efficacious regimen for treatment-naïve WM patients, with 5-year PFS rate of 65.5% (95%CI:48.8–77.9); these data are also similar to those of our study even if it includes RR patients.

A major concern with bortezomib treatment was a high rate of grade ≥3 peripheral neuropathy observed when bortezomib was given intravenously twice a week. Treon reported neurotoxicity in 7 (30%) of 23 patients treated with BDR regimen[14]; in B-DRC grade ≥3, adverse events occurred in 49.5% of patients and peripheral sensory neuropathy grade 3 occurred in two patients treated with B-DRC.[16]

In our study, by using subcutaneous weekly bortezomib, neurological toxicity rate was low, mainly of grade 1–2, and did not require treatment interruption.

In elderly patients with RR WM (median two prior lines of therapy), rituximab plus bendamustine (BR) determined 1-year and 3-year PFS of approximately 80% and 60% respectively.[6] With BR, prolonged myelosuppression was more common in patients who previously had received fludarabine or cladribine.[18] The rate of secondary neoplasm was lower than in previous study with fludarabine.[4]

In our study, we confirmed the high efficacy of BRB, with a 30-month PFS rate of 79% (95%CI 62–89) and an OS of 92% (95%CI 77%–97%). These data are similar to those obtained with BDR in untreated patients (median PFS of 42 months and 3-year OS of 81%)[16] and to those obtained with BR in RR WM (1-year PFS of 80%)[6] or with BTKi (median PFS at 3 years of 81% and 3-year OS of 85% with zanubrutinib).[15]

The ORR was 82%, including almost 50% of patients obtaining a VGPR or CR. Moreover, the depth of response improved during follow-up in four patients (10%). These data are similar to those obtained with purine analogues in first line.[19]

Moreover, the combination of bendamustine with rituximab and bortezomib was associated with a low extra-haematological toxicity and has the advantage of being a fixed duration therapy. Another benefit of bortezomib therapy is that it overcomes the negative prognostic impact of *CXCR4* mutations[20]; in our study, only two patients carried a *CXCR4* mutation and, as expected, this did not impact outcome.

On the other hand, BTK-i certainly showed a high response rates in both treatment-naive and RR WM[21,22]; however, in patients at risk for bleeding or cardiac complications, they may be poorly tolerated. In our study, despite 42% of patients had at least one comorbidity, mostly cardiovascular disease or metabolic disorders, the rate of extra-haematological toxicity was low (only one patient developed a cerebrovascular accident during the fifth cycle). For this reason, BRB seems to be safe even in patients with multiple comorbidities (including cardiological ones).

Moreover, during a long follow-up, BTK-i discontinuation rates for toxicity were about 15%[23] leading a 5-year PFS rate for all patients of 54%.[23] Therefore, in the RR therapeutic scenario, there might be still place for alternative high-effective, well-tolerated and fixed duration regimens such as BRB.

Nonetheless, we recognize the limitations of our study: First, the small number of patients and the long period of enrolment time due to the fact that BR had become usual first-line therapy and BTK-I could be used in clinical practice. Another limitation for our study is that ddPCR for biological study was standardized only after the study had started; thus, biological analysis was not performed in all patients. However, biological data offer interesting insights: Mutational data for *MYD88* and *CXCR4* mutations collected from 21 patients at baseline pointed out a good concordance between BM and cfDNA, moreover confirmed the risk of false-negative results when only PB of rituximab pretreated patients is analysed.[9]

We observed a deep MRD shrinkage after BRB, leading to a considerable rate of MRD negativity (ranging from 29% in BM to 75% in PB). Even if molecular remission was recently demonstrated as an independent PFS predictor in a retrospective series of WM patients,[11] as the current median follow-up (30 months) of our prospective evaluable series is still limited for an indolent disease, no statistically significant impact of MRD on clinical outcome was registered, so far.

In conclusion, the BRB regimen with bortezomib, rituximab and bendamustine provides a low-cost, fixed duration and efficient treatment with an acceptable and easily manageable toxicity in patients with pretreated WM, inducing high rates of clinical and MRD responses, along with remarkable PFS and OS rates and, thus, might be considered as an additional treatment option for patients with RR-WM.

AUTHOR CONTRIBUTIONS

GB conceived and designed the study, analysed and interpreted data; GB and SF conceived and designed the biological substudy; GB, SF and DD wrote the manuscript; AC provided statistical analysis; all authors collected and assembled data and approved the final version of the manuscript and are accountable for all aspects of the work.

AFFILIATIONS

[1]AOU Città della Salute e della Scienza di Torino—Ematologia U, Turin, Italy
[2]Ematologia U, Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, Università di Torino, Turin, Italy
[3]UOSD di Ematologia, ASL Roma 1, Rome, Italy
[4]SSD Epidemiologia Clinica e Valutativa—AOU Città della Salute e della Scienza di Torino e CPO, Piemonte, Italy
[5]AOSS Antonio e Biagio e Cesare Arrigo—SC Ematologia, Alessandria, Italy
[6]AOU Città della Salute e della Scienza di Torino—Ematologia, Turin, Italy
[7]Ospedale Businco—SC Ematologia e CTMO, Cagliari, Italy
[8]Presidio Ospedaliero A. Tortora—UO Onco-ematologia, Pagani, Italy
[9]PO San Martino—Ematologia, Oristano, Italy
[10]Ospedale Degli Infermi—SC Oncologia, Ponderano, Italy
[11]AOU Maggiore della Carità di Novara—SCDU Ematologia, Novara, Italy
[12]UOC Ematologia—Fondazione IRCCS Cà Granda OM Policlinico, Milan, Italy
[13]UOC di Ematologia, Azienda Os pedaliera Papardo, Messina, Italy
[14]Ospedale degli Infermi di Rimini—UO Ematologia, Rimini, Italy
[15]IRCCS-Centro di Riferimento Oncologico—UO di Ematologia e Trapianto Cellule Staminali, Rionero in Vulture, Rionero, Italy
[16]IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy
[17]Ospedale di Circolo—ASST Sette Laghi—UOC Ematologia, Varese, Italy
[18]Department of Clinical and Experimental Oncology, Veneto Institute of Oncology, Castelfranco Veneto, Italy
[19]Ospedale Santa Maria delle Croci—Ematologia, Ravenna, Italy
[20]Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

ACKNOWLEDGEMENTS

The authors would like to thank all patients who participated in the trial, the Fondazione Italiana Linfomi (FIL) data centre and the members of the Data Safety and Monitoring Board. The authors thank Silvia Zibellini for NGS analysis. The authors thank Lorella Orsucci and Umberto Vitolo for contributing to the drafting of the protocol. The authors thank all the local data managers, nurses and laboratory and pharmacy personnel for their essential assistance with collecting and managing the study data.

CONFLICT OF INTEREST STATEMENT

GB: speaker's bureau and advisory board: Janssen, BMS, GSK, Novartis, Menarini. SF: research funding: Janssen, Morphosys, Gilead, Beigene; consultancy: EusaPharma, Janssen, Sandoz, Abbvie; advisory Board: EusaPharma, Janssen, Clinigen, Incyte, Italfarmaco; speakers Honoraria: Janssen, EusaPharma, Servier, Gentili; research funding: Janssen, Beigene. CB: advisory board: ABBVie; speaker grant: Janssen. GG: advisory boards and speaker's bureau: Abbvie, Astra-Zeneca, BeiGene, Incyte, Hikma, Janssen, Lilly. FGR: advisory board: Janssen, Incyte, Takeda. GM: advisory and honoria: Janssen, Takeda, Incyte, Abbvie. RF: consultant: Kite, Incyte, Sobi, Novartis. MV: advisory board and speaker honoraria: ABBVie, Astrazeneca, Beigene: advisory board: Janssen. NV, AC, FM, DD, CC, LC, FC, AC, DD, TCdT, MF, DM, PT, GP, MM, RS, MT: no CO.

DATA AVAILABILITY STATEMENT

For clinical original data, please contact: gbenevolo@cittadellasalute.to.it. For biological original data, please contact: simone.ferrero@unito.it.

ETHICAL APPROVAL

The study was conducted according to the International Conference on Harmonization Guideline for Good Clinical Practice and to the Declaration of Helsinki. The protocol and its appendices were subject to review and approval by the competent Independent Ethics Committee(s).

PATIENT CONSENT STATEMENT

All patients provided written consent.

CLINICAL TRIAL REGISTRATION (INCLUDING TRIAL NUMBER)

This trial was registered at http://www.clinicalstrial.gov(NCT02371148) (EudraCT Number: 2013-005129-22).

DISCLAIMER

Bortezomib was provided by Janssen Pharmaceutical which did not have any influence on the analysis of the data or the interpretation of the results.

ORCID

Giulia Benevolo https://orcid.org/0000-0002-3200-9654
Carola Boccomini https://orcid.org/0000-0002-4155-403X
Federica Cavallo https://orcid.org/0000-0003-2047-1099
Michele Merli https://orcid.org/0000-0002-0905-5927
Marzia Varettoni https://orcid.org/0000-0001-7304-1629
Simone Ferrero https://orcid.org/0000-0002-9711-1502

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How to cite this article: Benevolo G, Drandi D, Villivà N, Castiglione A, Monaco F, Boccomini C, et al. Efficacy and safety of bendamustine, rituximab and bortezomib treatment in relapsed/refractory Waldenstrom Macroglobulinaemia: results of phase 2 single-arm FIL-BRB trial. Br J Haematol. 2024;00:1–9. https://doi.org/10.1111/bjh.19920