Bendamustine and rituximab as first-line treatment for symptomatic splenic marginal zone lymphoma: long-term outcome and impact of early unmeasurable minimal residual disease attainment from the BRISMA/IELSG36 phase II study

by Emilio Iannitto, Simone Ferrero, Côme Bommier, Daniela Drandi, Martina Ferrante, Krimo Bouabdallah, Sylvain Carras, Guido Gini, Vincent Camus, Salvatrice Mancuso, Luigi Marcheselli, Angela Ferrari, Michele Merli, Benoit Tessoulin, Caterina Stelitano, Kheira Beldjord, Giovanni Roti, Fabrice Jardin, Barbara Castagnari, Francesca Palombi, Lucile Baseggio, Alexandra Traverse-Glehen, Claudio Tripodo, Anna Marina Liberati, Margherita Parolini, Sara Usai, Caterina Patti, Massimo Federico, Maurizio Musso, Marco Ladetto, Emanuele Zucca, and Catherine Thieblemont

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Emilio Iannitto\textsuperscript{1,2}, Simone Ferrero\textsuperscript{3}, Côme Bommier\textsuperscript{4,5}, Daniela Drandi\textsuperscript{3}, Martina Ferrante\textsuperscript{3}, Krimo Bouabdallah\textsuperscript{6}, Sylvain Carras\textsuperscript{7}, Guido Gini\textsuperscript{8}, Vincent Camus\textsuperscript{9}, Salvatrice Mancuso\textsuperscript{10}, Luigi Marcheselli\textsuperscript{11}, Angela Ferrari\textsuperscript{12}, Michele Merli\textsuperscript{13}, Benoit Tessoulin\textsuperscript{14}, Caterina Stelitano\textsuperscript{15}, Kheira Beldjord\textsuperscript{4}, Giovanni Roti\textsuperscript{16}, Fabrice Jardin\textsuperscript{8}, Barbara Castagnari\textsuperscript{17}, Francesca Palombi\textsuperscript{18}, Lucile Baseggio\textsuperscript{19}, Alexandra Traverse - Glehen\textsuperscript{20}, Claudio Tripodo\textsuperscript{2}, Anna Marina Liberati\textsuperscript{21}, Margherita Parolini\textsuperscript{22}, Sara Usai\textsuperscript{23}, Caterina Patti\textsuperscript{24}, Massimo Federico\textsuperscript{25}, Maurizio Musso\textsuperscript{1}, Marco Ladetto\textsuperscript{26}, Emanuele Zucca\textsuperscript{27}, Catherine Thieblemont\textsuperscript{4,5}.

Affiliations

1 La Maddalena Department of Oncology, Onco-Hematology and Bone Marrow Transplantation Unit, Palermo, Italy.

2 Tumor Immunology Unit Department of Health Sciences University of Palermo School of Medicine. Istituto di Patologia Generale, Palermo, Italy.

3 Division of Hematology, Department of Molecular Biotechnologies and Health Sciences, University of Torino, Italy.

4 Université Paris Cité.


6 CHU de Bordeaux - Hôpital Haut Lévêque and Centre François Magendie, Service d'Hématologie Clinique et Thérapie Cellulaire, Pessac, France.

7 Univ. Grenoble Alpes. Institute For Advanced Biosciences (INSERM U1209, CNRS UMR 5309). University Hospital, Molecular Biology & Hematology departments, Grenoble, France.

8 Ematologia AOU delle Marche, Ancona, Italy.

9 Department of Hematology, Centre Henri Becquerel, Rouen, France.
Authors contributions

EI developed the study concept and design, analyzed and interpreted the data, and wrote the manuscript. CT, EZ, MF developed the study concept and design and interpreted the data. SF performed MRD investigations data analysis and wrote the manuscript; DD and MF performed
MRD investigations and contributed to data analysis; LB, ATG, and CLTR contributed to the study design and supervised the diagnostic process; LM performed statistical analysis; All authors provided materials or patients, collected, assembled, analyzed and interpreted the data, gave the final approval of the manuscript and are accountable for all aspects of the work.

Authors disclosures


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**Corresponding author**

Emilio Iannitto  
c.d.c. La Maddalena Department of Oncology, Onco-Hematology and Bone Marrow Transplantation Unit. Via San Lorenzo 312/d, 90146 Palermo Italy. Mobile +39 3274271454  Fax +39 091 6806814
Dear Sir,

Splenic marginal zone lymphoma (SMZL) is a rare histotype of non-Hodgkin lymphoma, accounting for only 2% of all cases. At diagnosis, up to 30% of patients may be asymptomatic, while others may present with cytopenia(s), abdominal lymph node swelling, splenomegaly, B-symptoms, and secondary autoimmune diseases. SMZL follows an indolent course that, similarly to other indolent lymphomas, can be complicated by the histological evolution into diffuse large B-cell lymphoma (DLBCL). After the diagnosis of SMZL, the risk of lymphoma-related death increases significantly, especially in patients who experience progression within the first 24 months. However, the 5-year conditional survival of the remaining patients is comparable to that of the general population, and the overall survival (OS) exceeds ten years.

Rituximab immunotherapy is a commonly used first-line treatment due to its effectiveness and mild toxicity. Although splenectomy is an effective procedure, it can lead to severe and potentially fatal acute and late complications. Additionally, the role of bendamustine-rituximab chemoimmunotherapy has yet to be defined, and the preferred first-line treatment is undetermined due to the lack of randomized studies.

It is unclear if the quality of clinical response, complete vs. partial (CR vs PR), correlates with the time-related outcomes. Additionally, insufficient evidence prevents evaluating the prognostic role of acquiring an undetectable minimal residual disease (MRD-) status. Given this context, the IELSG conducted the BRISMA-IELSG36 phase II study (EudraCT Number 2011-000880-28, NCT02853370). Approval by local ethics committees and written informed consent by all participants before study entrance was required. Through a series of 56 SMZL patients, the primary study objective aimed to assess the efficacy and toxicity of combining bendamustine with rituximab (BR) as a first-line therapy. Herein, we
present the updated results integrated with MRD data (median follow-up: 69 months; 95% CI 67-72).

Eligible patients needed to exhibit active/symptomatic disease, and the clinical responses scored according to the criteria described below, proposed by the Splenic Lymphoma Study Group (SLSG)\textsuperscript{10} for non-splenectomized patients: PR: $\geq 50\%$ improvement in the disease manifestations including resolution or decrease in spleen size, improvement on cytopenias and resolution or decrease in lymphadenopathy. Bone marrow (BM) should show a reduction in lymphoid infiltration. CR: resolution of organomegaly, normalization of the blood counts (Hb$>12$ g dl$^{-1}$; platelets$>100$ x 10$^3$ l$^{-1}$; neutrophils $>1.5$ x 10$^3$ l$^{-1}$ and no evidence of circulating clonal B-cells). No evidence of lymphoma in BM through immunohistochemistry. Table S1 compares the SLSG and Lugano response criteria for non-PET-avid NHL.

In addition, we adopted the term "unconfirmed complete response" (CRu).\textsuperscript{6,9} CRu describes patients who still exhibit some degree of cytopenia and splenomegaly at the end of treatment (EOT) but who subsequently meet the criteria for CR at the first follow-up visit.\textsuperscript{9} The MRD analysis was performed centrally in a EuroMRD standardized laboratory (https://euromrd.org/) by droplet digital PCR (ddPCR) with ASO primers targeting IGH rearrangements in the bone marrow (BM) and peripheral blood (PB) samples, expressed as copies out 250ng of genomic DNA as previously described.\textsuperscript{11} The MRD assay was considered reliable when reaching a sensitivity of at least $1\times10^{-4}$, calculated considering the tumor infiltration at baseline. Accordingly, in each ddPCR experiment, the \textquotedblleft10$^{-1}$\textquotedblright (as control) and the \textquotedblleft10$^{-4}$\textquotedblright dilution points were included.

Patients with a molecular marker underwent MRD assessment after three cycles (Early Restaging. ER) at EOT, and yearly at each annual follow-up (FU) assessment.

Clinical information has been updated for all living patients except for two. Presenting features are shown in Table S2. The median age was 66, and 41 patients (73\%) were
older than 60. Fifty-one patients (91%) achieved a major response (34 CR; 7 CRu; 10 PR). All the CRu patients and one in stable disease (SD) at EOT improved slowly and steadily, meeting the criteria for CR without additional treatment. Progression-free survival (PFS) events included three progressions documented in patients scored in SD at EOT, six relapses, and one death (due to sepsis at 1.6 months). According to the intention-to-treat analysis, the five-year PFS was 83% (95% CI 71-91%), and the OS was 93% (95% CI 82-97%) (Figure 1A-B). These results are nearly identical to those achieved with six weekly infusions of rituximab followed by 1-2 years of maintenance therapy.6 None of the ten patients in PR progressed during the median observation time of 80.9 months (range 64-103). At baseline, 53 out of 56 patients assessable for MRD had at least one FU sample and were screened for a monoclonal IGH rearrangement. A reliable MRD molecular marker was found in 42/53 (79%) patients, consistent with the literature.8 The ASO assay achieved adequate sensitivity for MRD analysis (at least 10^-4) in 37/53 (70%) cases. Among these 37 cases, 14 were identified in both BM and PB samples, 18 in PB only, and 5 in BM only (Figure 2B).

MRD data were available in 5 of 10 PR patients; at EOT, four were MRD-, and 1 was MRD+. Four out of 6 CR patients who relapsed had MRD EOT assessment: 3 were MRD+ and one MRD-. Figure 2A shows the clinical response, MRD status at different time points, and outcome for each of the 37 evaluable patients. The baseline MRD burden was not associated with presenting features, PFS, and OS (p >0.5).

Four patients died: one due to sepsis and three due to lymphoma at 1.6-27-40 and 42 months, respectively. Two cases of G>2 infections occurred during treatment,9 and three cases (2 acute bronchitis, one lung tuberculosis) occurred during the follow-up. 284 cycles were administered during the study, with 85% given at full dose. Bendamustine dose was reduced in four patients in cycles 4-6. Relative dose intensity was 0.99 for bendamustine and 0.98 for rituximab.
Seven patients developed a second primary cancer (SPC); the SPC includes one follicular thyroid cancer, one DLBCL, one renal-cell carcinoma, one basal cell carcinoma, one 5q-myelodysplastic syndrome, one malignant peripheral nerve sheath tumor, and one prostate adenocarcinoma. The 5-year cumulative incidence rate of SPC was 11%.

BR treatment resulted in high rates of undetectable MRD: 40% at the ER (17/37), 54% (14/26) at the EOT, and 65% (20/31) after one year (Figure 2B). Interestingly, a significant reduction in MRD had already been attained at ER assessment (median 4.7E-05 in BM and below the quantitative level in PB, respectively). The additional BR courses (one in 3 CR patients and three in 9 PR patients) did not contribute to a significant further reduction in residual MRD levels (median 1.4E-05 in BM and BQL in PB, respectively) (Figure S1 A-B). MRD+ predicted a statistically significant inferior PFS at each evaluated timepoint (ER, EOT, and FU1) (Figure 3 A-B-C). Notably, acquiring early MRD- status in non-invasive peripheral blood samples was associated with a significant improvement in PFS (MRD- vs. MRD+ 100% vs 68% (95CI 35-86%) p =0.006 (Figure 3D).

Our analysis of MRD assessment aligns with published data for rituximab-chemo treated SMZL patients.7-8 Cervetti et al. generated MRD data assessing by real-time quantitative polymerase chain reaction (RQ-PCR) IgH rearrangements in a retrospective series of 50 SMZL (EOT MRD- 48%; PFS:100% versus 73% p = 0.023).8 Lyu et al. investigated MRD status by employing multi-colour flow cytometry (MFC) in a prospective series of 71 patients (EOT MRD- 77%; PFS:74.8 ± 6.5% vs 31.4 ± 12.6%, P < 0.001).7 In our study, the EOT MRD status in a landmark analysis was significantly associated with PFS (Figure 3B), while no difference in PFS was observed between CR and PR groups (p=0.438 not-shown). Three MRD+ patients slowly converted to unmeasurable MRD- status, and 7 CRu and one SD patient eventually met the criteria for CR at FU1 12 months after completion of treatment. None of the patients received off-protocol treatments or maintenance therapy. A speculative explanation for the spontaneous conversion to MRD- might be the gradual
elimination of minimal subsets of residual neoplastic cells by the restored activity of the immune system after EOT. Accordingly, the MRD levels of these three patients were below 1x10^-4 (namely 2, 2, and 3 copies/50000 cells). In conclusion, the BRISMA study substantiates that BR chemoimmunotherapy accomplishes a swift and significant reduction in tumor bulk for patients with symptomatic SMZL, thereby facilitating notably high 5-year PFS and OS rates. Other retrospective studies have reported comparable PFS and OS rates with less toxic treatments.\textsuperscript{6;13} However, this phase II study with extended follow-up provides compelling evidence for considering bendamustine-rituximab as an effective treatment for symptomatic SMZL patients.

Two other informative results come from the MRD analysis: 1) Our findings support the assertion that attaining MRD- after just three courses of treatment is a promising indicator of favorable long-term outcomes, even when monitored in PB.\textsuperscript{7-8;12} Though data are not yet mature enough to use MRD status in clinical decisions, it could help stratify patients in future trials. Furthermore, integrating MRD results with response criteria is worth exploring due to the imaging limitations in detecting the disease in the spleen.\textsuperscript{14} Finally, the incidence of additional cancers in this series aligns well with the cumulative incidence rate documented in existing literature.\textsuperscript{15} Consequently, we recommend conducting an SPC assessment before initiating a BR treatment and throughout the follow-up period, particularly in elderly patients.
References


Legend

Figure 1
Progression Free Survival and Overall Survival @ 5 years. Median observation time 69 months.
A) PFS: Progression-Free Survival; B) OS: Overall Survival.

Figure 2
Clinical Response and undetectable Minimal Residual Disease rate at different time points.
A) Individual MRD status, clinical response, and outcome at different times. B) Undetectable MRD rate at different time points.
MRD: minimal residual disease; ER: Early restaging at 4 months; EOT: end of treatment at six months; FU-1: one-year follow-up; FU-2: two-year follow-up; Last -FU: last available control. Red Spot: MRD+ (at least one MRD positive sample, either bone marrow or peripheral blood) Blu spot: MRD-; (unmeasurable MRD); Black spot: dead; CR: complete remission; PR: partial remission. SD: stable disease; R: relapse; P: progression; Green arrow: alive at last follow-up; BM: bone marrow; PB: peripheral blood.

Figure 3
Landmark PFS analysis stratified by MRD evaluated at different time points.
A) Overall MRD at ER; B) Overall MRD at EOT; C) Overall MRD at FU-1; D) Peripheral blood MRD at ER.
PFS: progression-free-survival; MRD: minimal residual disease; Overall MRD: at least one MRD+ positive sample (either bone marrow or peripheral blood); MRD-: unmeasurable MRD; ER: early restaging after 3 bendamustine rituximab courses; EOT: end of treatment; FU-1, one-year follow-up.
Figure 1

A) PFS

5-yr PFS 83% (95%CI 71-91%)

B) OS

5-years OS 93% (95%CI 82-97%)
**Supplement File Legends**

**Table S 1**

**SLSG response criteria for SMZL and Lugano 2014 response criteria for non-Pet avid NHL**

SMZL: splenic marginal zone lymphoma; SLSG: splenic lymphoma study group; NR: no-response/progression; CR: complete remission; PR: partial remission; IHC: immunohistochemistry; PPD: cross product of the longest transverse diameter of a lesion and perpendicular diameter; SPD: sum of the product of the perpendicular diameters for multiple lesions.

**Table S 2**

**Presenting and demographic data on diagnosis.**


**Figure S1 A**

**MRD shrinkage in bone marrow assessed at different time points.**

Abbreviation. MRD levels at different time points measured by ddPCR and expressed as copies out of 250ng of genomic DNA. Black lines represent the median MRD level at each time point: DIA 1.3E-01 (range: 1.3E-03 -1.7), ER 4.7E-05 (range: BQL – 2.3E-01); EOT 1.4E-05 (range: BQL – 6.7E-05), FU1 1E04 (range: BQL – 8.1E-01). Abbreviations: MRD minimal residual disease; DIA, diagnosis; ER, early restaging; EOT end of treatment; FU1, one-year follow-up; BQL, positive below quantity limit value; NEG, negative; R, represents a patient who will relapse in the subsequent FU.

**Figure S1 B**

**MRD shrinkage in peripheral blood assessed at different time points.**

Abbreviation. MRD levels at different time points measured by ddPCR and expressed as copies out of 250ng of genomic DNA. Black lines represent the median MRD levels level at each time point: DIA 2.7E-01 (range 2.8E-03 – 1.4E+00), ER BQL (range BQL – 3.9E-01), EOT BQL (range: BQL – 4E-05), FU1 5.3E-05 (range BQL -8.8E-01). Abbreviations: MRD minimal residual disease; DIA, diagnosis; ER, early restaging; EOT end of treatment; FU1, one-year follow-up; BQL, positive below quantity limit value; NEG, negative; R, represents a patient who will relapse in the subsequent FU.
### Table S 1

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| **CR**           | ➢ Resolution of organomegaly  
➢ Hb > 12 g dl  
➢ Platelets > 100,000/mmc  
➢ Neutrophils > 1500/mmc  
➢ No evidence of circulating clonal B cells  
➢ No evidence or minor BM infiltration detected by immunohistochemistry | Complete radiologic response  
➢ Spleen < 13 cm in vertical length  
➢ Nodal masses < 1.5 cm  
➢ Bone marrow normal by morphology; if indeterminate, IHC negative |
| **PR**           | >50% improvement; this should include:  
➢ Resolution or decrease of spleen size  
➢ Improvement on cytopenias  
➢ Decrease in lymphadenopathy  
➢ Decrease of BM infiltration | All the following:  
➢ Spleen must have regressed by > 50% in length beyond normal  
➢ >50% decrease in SPD of up to 6 target measurable nodes and extranodal sites  
➢ Bone marrow : not applicable |
| **NR**           | Less than 10% improvement on the disease manifestations or deterioration of the above, respectively. | Spleen: increase by 50% of the extent of the prior size or if normal, > 2 cm from the baseline  
Nodes: Increase by > 50% from PPD nadir. |
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