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A phase 2 study of rituximab, bendamustine, bortezomib and dexamethasone for first-line treatment of older patients with mantle cell lymphoma

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ABSTRACT

We present results of a prospective, multicenter, phase II study evaluating rituximab, bendamustine, bortezomib and dexamethasone as first-line treatment for patients with mantle cell lymphoma aged 65 years or older. A total of 74 patients were enrolled (median age, 73 years). Patients received a maximum of six cycles of treatment at 28-day intervals. The primary objective was to achieve an 18-month progression-free survival rate of 65% or higher. Secondary objectives were to evaluate toxicity and the prognostic impact of mantle cell lymphoma prognostic index, Ki67 expression, [18F]fluorodeoxyglucose-positron emission tomography and molecular minimal residual disease, in peripheral blood or bone marrow. With a median follow-up of

52 months, the 24-month progression-free survival rate was 70%, hence the primary objective was reached. After six cycles of treatment, 91% (54/59) of responding patients were analyzed for peripheral blood residual disease and 87% of these (47/54) were negative. Four-year overall survival rates of the patients who did not have or had detectable molecular residual disease in the blood at completion of treatment were 86.6% and 28.6%, respectively (P<0.0001). Neither the mantle cell lymphoma index, nor fluorodeoxyglucose-positron emission tomography nor Ki67 positivity (cut off of ≥30%) showed a prognostic impact for survival. Hematologic grade 3-4 toxicities were mainly neutropenia (51%), thrombocytopenia (35%) and lymphopenia (65%). Grade 3-4 non-hematologic toxicities were mainly fatigue (18.5%), neuropathy (15%) and infections. In conclusion, the tested treatment regimen is active as frontline therapy in older patients with mantle cell lymphoma, with manageable toxicity. Minimal residual disease status after induction could serve as an early predictor of survival in mantle cell lymphoma. *ClinicalTrials.gov: NCT 01457144.*

Introduction

Mantle cell lymphoma (MCL) is a rare subtype of B-cell non-Hodgkin lymphoma characterized by the genetic hallmark t(11;14)(q13;q32) chromosomal translocation which leads to overexpression of cyclin D1.[1] The standard-of-care for the treatment of older MCL patients (>65 years), has been eight cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) given at 21-day intervals (R-CHOP-21), followed by maintenance therapy which has been shown to improve response duration and overall survival (OS) in patients who reach the maintenance phase.[2] Complete response rates do, however, remain low with R-CHOP (30-35%) and the median progression-free survival (PFS) is in the range of 14-18 months.[3,4] After R-CHOP and maintenance therapy, the 4-year OS rate was 87%.[2] Although dose-intensive and high-dose cytarabine-containing regimens, with or without autologous stem cell transplantation consolidation in younger patients, has improved outcomes (the median PFS is now well in excess of 5 years), such approaches are frequently not feasible, given that the median age at diagnosis of MCL is in the mid to late 60s.[1]

Bortezomib was the first novel agent to be approved for the treatment of patients with relapsed/refractory MCL.[5,6] The addition of bortezomib to rituximab-anthracycline-based regimens has improved the results, compared to those achieved by R-CHOP, for frontline therapy in MCL, leading to a complete response rate of 50% and a median PFS of 25 months albeit with increased hematologic toxicity.[7,8] Two phase III trials have shown the superiority of bendamustine-rituximab combination therapy over R-CHOP or R-CHOP/R-CVP (rituximab, cyclophosphamide, vincristine, prednisolone) with respect to overall and complete response rates and reduced toxicity.[9,10] However, superior PFS was observed in only one of the latter phase III studies.[9] More recently, combining genotoxic agents (such as cytarabine) or targeted agents (such as bortezomib or lenalidomide) with bendamustine and rituximab (BR) has shown efficacy in both first-line and salvage therapy in MCL.[1,11-13]

In anticipation of the above findings, our group initiated a phase II trial to assess the efficacy of a new regimen combining rituximab, bortezomib, bendamustine and dexamethasone (RiBVD) for first-line therapy of older MCL patients. Specifically, for the trial design, associating RiBVD, we took into account the interim results of the BR regimen, for which the reported overall response rates were 90% in relapsing MCL patients,[14,15] and the promising results (32% overall response rate) of bortezomib monotherapy in relapsing MCL patients (PINNACLE study).[6] Pre-defined secondary objectives of our study included assessment of molecular complete response rates in blood and bone marrow and evaluation of their prognostic impact on survival.

Methods

Study design and patients

The RiBVD multicenter phase II trial enrolled newly diagnosed MCL patients ≥65 years or <65 years if ineligible or unwilling to undergo autologous stem cell transplantation. The study was conducted in 37 centers of the Lymphoma Study Association (LYSA) (NCT 01457144) and was approved by institutional review boards and ethics committees at all sites, and conducted according to the Declaration of Helsinki. The diagnosis of MCL was established according to the World Health Organization (WHO) 2008 criteria. Ki67 staining and scoring were performed centrally, according to European MCL Network recommendations.[16] All pathology results were reviewed centrally by the LYSA pathology commission. Eligible patients gave written, informed consent, as per standard guidelines. Inclusion and exclusion criteria are summarized in *Online Supplementary Table S1*.

The RiBVD regimen consisted of a maximum of six cycles of 28 days each for all enrolled patients, as described in the *Online Supplementary Methods* and *Online Supplementary Table S2*.

Response and safety assessments

The International Working Group (IWG) 1999 and 2007 criteria were used to define responses after four and six cycles, respectively. [^{18}F]fluorodeoxyglucose (FDG)-positron emission tomography (FDG-PET) responses were evaluated in each center with the five-point scale, visual method of Deauville.[17] Hematologic and non-hematologic toxicity was monitored continuously during treatment and at follow-up visits and graded according to the National Cancer Institute criteria (Common Terminology Criteria for Adverse Events, version 3.0) (see the *Online Supplementary Methods* for details).

Molecular minimal residual disease

Molecular responses were evaluated centrally by real-time quantitative polymerase chain reaction targeted to patient-specific, *IGH V(D)J* clono-specific rearrangements, to quantify tumor B cells, according to EURO-MRD guidelines, as previously described.[18] Minimal residual disease (MRD) analysis was per-

formed before treatment (baseline), after four courses of treatment (mid-term MRD), and at the end of treatment (after 6 courses of RiBVD) in peripheral blood and bone marrow until progression or relapse, for a maximum follow-up period of 3 years.[18,19] During the follow-up, MRD was evaluated in the blood at 3 monthly intervals for 1 year and every 6 months thereafter while bone marrow MRD monitoring was performed at yearly intervals. A description of additional methods, the MRD study cohort, sample source and numbers is given in the *Online Supplementary Methods* and illustrated in *Online Supplementary Figure S1*.

Sample size calculation and statistical analysis

The primary objective of the study was to prolong PFS by 6 months compared to the 18-month median PFS reported for patients treated with R-CHOP-21.[3] The number of patients to be enrolled was calculated by a one-step Fleming method. In order to define superiority of the RiBVD regimen over R-CHOP, a PFS rate of 65% or more (H1) was required at 18 months. The treatment was to be considered a failure if the PFS rate at 18 months was ≤50%. Taking into account alpha and beta risks of 5% and 20%, respectively, 69 patients needed to be enrolled. Based on a maximum 10% error in diagnosis, 76 patients had to be enrolled. Additional details are given in the *Online Supplementary Methods*.

Results

Patients

A total of 76 MCL patients were enrolled between November 2011 and December 2012 (Figure 1). All patients were monitored for 3 years after their last cycle of therapy. Two patients were excluded - one because of a misdiagnosis of MCL (diffuse large B-cell lymphoma) and one because of exclusion criteria (hepatitis B) – leaving 74 patients for data analyses (Figure 1). Seventy-one patients had MCL confirmed by central review. The diagnosis was made on tumor biopsies (45 on lymph nodes and 26 on extra-nodal tissue). Due to unsuccessful tissue biopsy in three patients, a diagnosis of MCL was made by flow cytometry in peripheral blood (1 patient) or bone marrow (2 patients). Ki67 staining was performed in 56 patients, and was found ≥30% positive in 59% of these patients (31 of 56 patients) (Table 2).

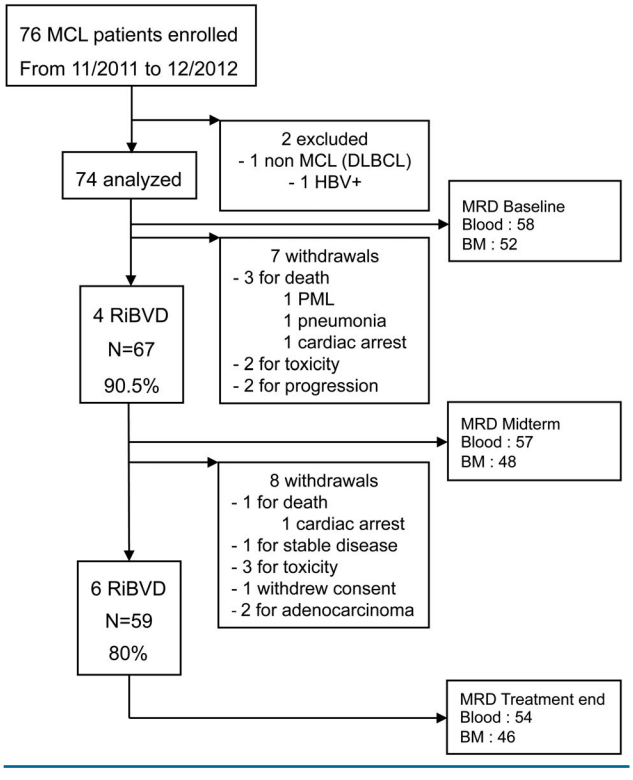

Figure 1. Consort diagram for the RiBVD phase 2 trial. MCL: mantle cell lymphoma; DLBCL: diffuse large B-cell lymphoma; HBV: hepatitis B virus; MRD: minimal residual disease; BM: bone marrow; PML: progressive multifocal leukoencephalopathy.

Treatment response

Seventy-four patients initiated therapy. Sixty-seven patients received at least four cycles (90.5%) of treatment

Table 1. Patients' demographics and clinical characteristics.

Characteristics	N.	%
Age (years)		
median	73	
range	64-83	
Sex		
male	49	66
female	25	34
WHO Performance Status		
0-1	73	85
2-4	11	15
Lactate dehydrogenase		
normal	44	61
>normal	28	39
B symptoms		
no	56	76
yes	17	24
Ann Arbor stage		
II	4	6
III-IV	70	94
Bulky tumor		
no	52	71
yes	21	29
Extranodal involvement		
no	7	9
yes	67	91
Bone marrow involvement		
no	24	34
yes	46	66
Spleen involvement		
no	38	52
yes	35	48
MIPI score		
low	2	3
intermediate	12	17
high	58	80
MIB1 / ki67 prolferation index		
<30%	21	41
≥30%	30	59
Pathology		
classic	61	86
blastoid	10	14

WHO: World Heath Organization; MIPI: Mantle Cell Lymphoma International Prognostic Index.

and 59 (80%) received all six planned cycles (Figure 1). Of the planned 444 cycles, 406 (91.5%) were administered. Fifteen patients stopped therapy before receiving all six cycles (Figure 1). After four cycles, the overall response rate was 86.5% (64/74) and the complete response rate (confirmed and unconfirmed complete responses) was 56.5% (42/74). At the end of treatment, the overall response rate was 84% (62/74) and the complete response rate was 75.5% (56/74). FDG-PET evaluations were performed after four cycles of treatment in 64 patients (100% of the 64 responders) and after cycle 6 in 59 patients (95% of the 62 responders). Interim and final FDG-PET were negative in 64% (41/64) and in 78% of evaluated patients (46/59), respectively.

Molecular minimal residual disease in blood and bone marrow

Molecular MRD was assessed in a total of 58 of the 74 patients eligible for MRD analysis (in all, 732 samples were assessed, see *Online Supplementary Figure S1*). Molecular MRD analysis was not possible in 16 of 74 MRD-eligible patients because of a lack of MRD target (n=6), missing follow-up samples (n=9) or because an MRD target reference sample was not available (n=1) (*Online Supplementary Figure S1*). After four cycles (mid-term), 57 patients were analyzed for molecular MRD (57 peripheral blood samples; 48 bone marrow samples, of which 48 patients with paired bone marrow and peripheral blood MRD samples, at the mid-term analysis). Of these, 50 patients were negative for molecular MRD (32 in complete remission, 18 in partial remission) and seven were positive (2 in complete remission, 4 in partial remission and 1 with stable disease) in the blood and/or bone marrow, for a molecular response rate of 79% (defined by a quantitative polymerase chain reaction assay with a sensitivity of 10^{-5}). After six cycles of treatment, 54 patients were analyzed for molecular MRD (54 peripheral blood samples; 46 bone marrow samples, of which 46 patients with paired bone marrow and peripheral blood samples for MRD analysis at the end of treatment). Of these 54 patients, 41 were MRD-negative (39 in complete remission, 1 in partial remission and 1 with stable disease) and 13 were MRD-positive (8 in complete remission, 4 in partial remission and 1 with progressive disease) in blood and/or bone marrow (76% molecular response rate) (Figure 2 and *Online Supplementary Figure S1*).

Molecular MRD response rates were then analyzed separately in the peripheral blood *versus* bone marrow at the mid-term follow-up time-point (after 4 treatment cycles) and at the end of treatment (after 6 cycles). Blood samples were molecular MRD-negative from 88% (50/57) of patients after four cycles and 87% (47/54 patients) after six cycles (Figure 2C, left panel). The corresponding percentages for bone marrow samples were 77% (37/48 patients) after four cycles of treatment and 76% (35/46 patients) at the end of treatment (after 6 cycles) (*Online Supplementary Figure S3*).

Survival analyses and prognostic factors

With a median follow-up time of 52 months, 74 patients were evaluable. Overall, 24 patients died, four during treatment (2 from cardiac arrest, 1 with pneumonia and 1 with progressive multifocal leukoencephalopathy, after cycle 3) and 20 during follow-up (16 due to progressive disease, 1 from pancreatic adenocarcinoma, 1 from cardiac arrest and 2 from unknown causes).

The 2-year PFS was 70.3% compared to 57.6% at 4 years. The 2-year OS was 81.1% compared to 71.3% at 4 years. The Mantle Cell Lymphoma International Prognostic Index (MIPI) score was not predictive for PFS or OS perhaps due to the small number of patients because a trend could be observed (Table 2 and *Online Supplementary Figure S2A*). Indeed, the 4-year OS for the 58 MIPI high-risk patients was 66.8% compared to 85.7% for the 14 MIPI low- or intermediate-risk patients (P=0.13). Neither histology (classical subtype *versus* blastoid subtype) nor Ki67 expression (<30% *versus* ≥30% of positive MCL cells in the tumor biopsy) was predictive for OS (P=0.10 and P=0.24, respectively) or PFS (P=0.08 and P=0.13, respectively) (Table 2 and *Online Supplementary Figure S2B*). Clinical responses (complete or partial response *versus* no response), as assessed by the Cheson 1999 criteria, were

Table 2. Prognostic factors for progression-free survival and overall survival.

Prognostic factors	N.	*P* for PFS	*P* for OS
Pathology (classic *vs.* blastoid form)	71	0.08	0.10
MIPI score (high *vs.* low/Intermediate)	72	0.18	0.13
Ki67 (< *vs.* ≥ 30%)	51	0.35	0.24
Response IWC 1999 (CR *vs.* PR *vs.* failure)	74	<0.0001	<0.0001
FDG-PET midterm	64	0.19	0.57
FDG-PET treatment end.	59	0.48	0.98
MRD blood and/or bone marrow at mid-term (neg 45; pos 12)	57	0.20	0.33
MRD blood and/or bone marrow at treatment end (neg 41; pos 13)	54	0.04	0.02
MRD blood mid-term (neg 50; pos 7)	57	0.01	0.047
MRD blood treatment end (neg 47; pos 7)	54	<0.0001	<0.0001
MRD bone marrow midterm (neg 37; pos 11)	48	0.24	0.41
MRD bone marrow treatment end (neg 35; pos 11)	46	0.20	0.19

N: number of patients who could be evaluated ; MIPI score,: Mantle-Cell Lymphoma International Prognosis Index; Ki67/Mib1, proliferation index score; Response IWC 1999, response according to the 1999 International Workshop Criteria; PF: progression free survival; OS: overall survival (at 4 years), respectively; CR: complete response; PR: partial response; FDG-PET, [18F]fluorodeoxyglucose positron emission tomography; mid-term, analysis after four cycles; treatment end, analysis after six cycles; MRD, (molecular) minimal residual disease.

highly predictive for PFS or OS whether determined at the mid-term staging or at the end of treatment. There were no survival differences (PFS or OS) between patients in partial or complete remission. Neither mid-term nor final FDG-PET scan responses were predictive for PFS or OS. The most highly predictive factor for PFS and OS (P<0.0001) was MRD status in peripheral blood at the end of treatment (Figure 2C, right panel and Table 2). Molecular blood MRD status at mid-term was also significant for PFS (P=0.01) and weakly significant for OS (P=0.047) (Table 2). By contrast, MRD status in the bone marrow after four cycles of treatment (mid-term) or at the end of treatment was not predictive for either PFS or OS (see *Online Supplementary Figure S3* for end-of-treatment data). The 4-year OS for patients who were MRD-negative in blood at the end of treatment (n=47/54) was 86.6% compared to 28.6% for blood MRD-positive patients (n=7/54).

Continued molecular remission status in the peripheral blood after therapy (at the 12-month follow-up) was significantly associated with longer PFS (33 patients; 4-year PFS 97%). By contrast, the median PFS for patients who remained MRD-positive (n=6) or who had converted to an MRD-positive status in the peripheral blood by the 12-month follow-up (n=7) was 11 and 26 months, respectively.

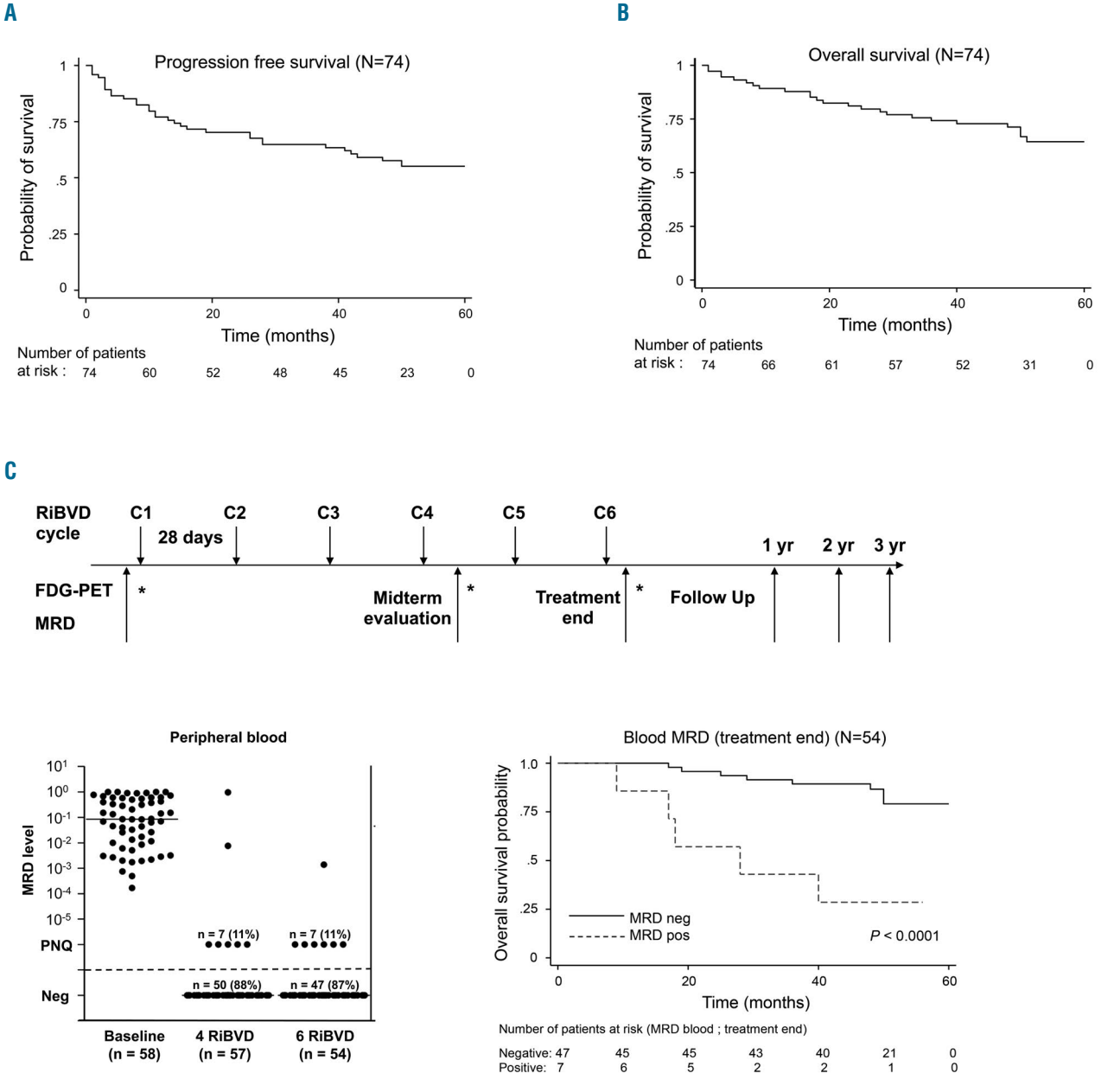

Figure 2. Survival of patients with mantle cell lymphoma following frontline treatment with the RiBVD regimen. (A) Progression-free survival of the 74 patients. (B) Overall survival of the 74 patients (C) Molecular response rates and overall survival according to molecular residual disease (MRD) status in peripheral blood after six cycles of RiBVD (treatment end).

Toxicity

Fifteen patients of 74 (20%) stopped treatment before the sixth cycle, four because of death (1 case each of pneumonia and progressive multifocal leukoencephalopathy and 2 cardiac arrests), five because of grade 3-4 toxicity [septicemia (n=1), neuropathy (n=2), digestive tract toxicity (n=1) and pleural effusion (n=1)], three because of progression or stable disease and three for other causes (Figure 1). During treatment, 49 of 74 patients (66.2%) developed grade 3-4 hematologic toxicities (51% neutropenia, 35% thrombocytopenia and 19% anemia) (Table 3). Neutropenia translated into febrile neutropenia in 11 patients (11%), which was grade 3-4 in six. Lymphopenia at the end of treatment was reported in 65% of the patients (48/70) and was mainly grade 3-4 (lymphocytes $<0.5x10^9$/L) (Table 3). Persistent grade 3-4 lymphopenia was seen in 28.8% of patients at 1 year after the completion of treatment (17 of 59 surviving patients who could be evaluated). Forty-two patients (56.7%) had non-hematologic grade 3-4 toxicities at the end of treatment (Table 3). The most frequent non-hematologic toxicities (seen in more than 10% of patients) were fatigue, peripheral neuropathy and fever with or without neutropenia, which occurred in 18.5% (n=14), 15% (n=11) and 15 (n=11) of cases, respectively (Table 3). Other toxicities were reported in four or fewer patients (i.e. less than 6%) and were as follows: pulmonary toxicity, cardiac toxicity, hyperglycemia, elevated transaminases, digestive tract toxicity, cutaneous rash, allergy and fever without neutropenia. No patient experienced cytomegalovirus reactivation or pneumocystis infection.

Twenty-four episodes of infection were declared as serious adverse events. These represented one-third of the 76 serious adverse events reported during the 406 cycles of therapy, or during follow up. They included seven cases of opportunistic infection (4 cases during treatment and 3 further cases 1 year after the end of treatment), which were as follows; herpes zoster (n=3), progressive multifocal leukoencephalopathy (n=1), cytomegalovirus colitis (n=1), listeriosis (n=1) and oral candidosis (n=1). Additional infections were pneumonia (n=9), staphylococcal infection (n=2), followed by non-recurring infections of various types (no more than 1 case each, as follows; acute pyelonephritis, bronchitis, catheter site infection, upper aero-digestive tract infection and *Clostridium difficile*-induced colitis).

Regarding neurotoxicity, grade 2 to 4 neuropathy was observed in 21.5% of patients (16 of 74 patients) (Table 3). Neuropathy was generally reported after cycle 3 of treatment. Bortezomib was stopped indefinitely in ten of the 11 patients with grade 3-4 neurotoxicity but not in cases of grade 2 toxicity. Partial reversibility of neuropathy was reported in 13 of the 16 patients (81%) with grade 2 to 4 neurotoxicity.

Discussion

We report the results of a prospective, phase II study by the French LYSA group. The study aimed to test the efficacy of six cycles of RiBVD, without maintenance therapy, for first-line treatment of MCL patients aged ≥65

Table 3. Hematologic and non-hematologic toxicity.

	All Grades		Grade 3		Grade 4	
Events	N.	%	N.	%	N.	%
Neutropenia	52	70	17	23	21	28.5
Thrombocytopenia	67	90.5	18	24	8	11
Lymphopenia	67	95.7	38	54	8	11
Anemia	70	94.5	6	8	0	0
Fatigue	56	75.5	12	16	2	2.5
Neuropathy	32	43*	10	13.5	1	1.5
Fever	35	47	5	6.5	2	2.5
Febrile neutropenia	8	11	4	5.5	2	2.5
Lung	26	35	4	5.5	2	2.5
Cardiac	16	21.5	4	5.5	1	1.5
Hyperglycemia	23	31	4	5.5	0	0
Rash	25	34	3	4	0	0
GOT/GPT	29	39	3	4	0	0
Digestive tract	35	47	3	4	0	0
Allergy	24	32	1	1	0	0
Weight loss	25	34	0	0	0	0
Nausea	25	34	0	0	0	0
Bilirubin	12	16	0	0	0	0
Creatinine	24	32.5	0	0	0	0
Ear	2	3	0	0	0	0
Infusion-related reaction	18	24	0	0	0	0
Calcium	4	5	0	0	0	0

GOT/GPT: glutamic oxaloacetic transaminase/glutamic-pyruvic transaminase; CMV: cytomegalovirus. *includes grade 2 = 6.8% (n=5/74).

years. With a median follow up of 52 months, the 2-year PFS of the 74 patients with analyzable data was 70%, thus reaching the primary objective of the study which was to improve median PFS by 6 months compared to the reported 18-month PFS for patients treated with R-CHOP.[3,4] The 4-year PFS (57.6%) observed here for RiBVD-treated patients is in line with the median PFS reported for the BR regimen (35.4 months) and other bortezomib-containing regimens such as 24.7 months for VR-CAP (bortezomib, rituximab, cyclophosphamide, doxorubicin, and prednisone) and 26 months for the RiPAD+C regimen (rituximab, bortezomib, doxorubicin, dexamethasone, and chlorambucil).[7-9] The favorable PFS with the RiBVD regimen may be related to the marked depth of response (75.7% rate of confirmed and unconfirmed complete responses according to the Cheson 1999 criteria; 78% rate of complete responses according to the Cheson 2007 criteria). Although not strictly comparable outside of a randomized trial, it is worth noting that complete response rates with other regimens are lower: R-CHOP (34%), BR (40%), VR-CAP (53%) and RiPAD+C (51%).[2,3,7,8] The higher rate of complete responses (confirmed and unconfirmed) with the RiBVD regimen translated into higher molecular response rates [76% of patients (41/54) were MRD-negative in blood and/or bone marrow at the end of treatment] compared to published data for R-CHOP in older MCL patients [67% MRD negativity (54/81 patients)].[20] It is worth noting that 80% of patients in our study had high-risk MIPI scores and that 59% had ≥30% Ki67 positivity (range, 5% to 95% positivity) which appears high compared to the percentages in other studies in MCL patients over 65 years old (see *Online Supplementary Table S4*).

In keeping with results of high-dose cytarabine treatment in younger MCL patients,[21-23] a recent phase II trial from an Italian group (FIL) confirmed the efficacy of the R-BAC500 regimen (which associates rituximab, bendamustine and cytarabine) for treatment of older MCL patients.[12] In updated clinical results for the R-BAC500 regimen, complete response rates of 93% and molecular response rates of 78% (35/45) in the blood have been reported.[24] The 2-year PFS and OS were estimated as 83% and 86%, respectively.[24] However, it is worth noting that the baseline characteristics of the MCL cohort treated with R-BAC500, the patients in our study and those in other published series of MCL cases differ quite widely (*Online Supplementary Table S4*).

The rate of treatment discontinuation in our study was 20% (15/74 patients). This is broadly in line with rates reported for R-CHOP [17% (43/242)] and VR-CAP [18.8% (45/240)] in older MCL patients treated in first line,[7] and is lower than that reported with the R-BAC500 regimen (33%)[24] (*Online Supplementary Table S4*). The reported rates of premature therapy cessation for the BR and R-CHOP regimens are 8% and 5%, respectively.[10]

The 5.4% rate of toxic deaths reported here with the RiBVD regimen is in line with that observed for other first-line regimens used in older MCL patients. For instance, in a phase III study comparing the R-CHOP regimen to VR-CAP, Robak and colleagues reported 14/242 deaths (6%) and 11/240 deaths (5%), in the R-CHOP and VR-CAP arms, respectively.[7] Of these deaths, a total of six were due to infection and three to cardiac failure.

A trial of lenalidomide, bendamustine and rituximab recently documented a complete response rate of 64%, with molecular MRD negativity reached in 34% patients. Toxicity, however, was greater than with the RiBVD regimen, with infectious grade 3-5 toxicities seen in 42% of the 51 recruited patients.[13] Two large phase III trials of BR ± ibrutinib or BR ± ACP196 (acalabrutinib) are still ongoing and results are pending.

Grade 3-4 hematologic toxicities observed with the RiBVD regimen (51% neutropenia and 35% thrombocytopenia) were in line with those seen in patients treated with other regimens such as R-CHOP (60% neutropenia and 18% thrombocytopenia), VR-CAP (85% and 57%, respectively) or R-BAC (rituximab, bendamustine and cytarabine; 49% and 52%, respectively).[2,7,24]

Lymphopenia (65% of grade 3-4), which is known to occur with the BR regimen, may contribute, with neutropenia, to the relatively high number of infectious episodes seen in this study.[9,10] The rate of lymphopenia at 1 year was 32.5%, which is indicative of longer-term immunosuppression with the RiBVD regimen. Whether this is related to the use of dexamethasone or to the immunosuppressive effects of bendamustine remains to be established but indicates that precautionary measures to control infection are advisable.

Contrary to published results for subcutaneous administration of bortezomib, we noted a relatively high incidence of grade 3-4 neurotoxicity, which is a limiting factor for the RiBVD regimen.[25] Although the incidence was comparable to that observed in our previous RiPAD+C trial (18% grade 3-4 toxicity, 7/39 patients), in which bortezomib was administered intravenously at the same dose,[8] it is higher than that observed in other studies using comparable intravenous doses in which grade 3-4 toxicity was reported in 7% to 8% of patients treated with R-BV (rituximab, bendamustine, and bortezomib) and VR-CAP regimens.[7,11] Further investigations will be required to understand the reason for this. Of note in this respect is the discovery of genetic risk loci for severe peripheral neuropathy in European patients with multiple myeloma treated with bortezomib.[26]

In this study, molecular response in peripheral blood at the end of treatment (after 6 RiBVD cycles) was identified as a major predictive factor for PFS and OS, thus further emphasizing the importance of the depth of response, beyond standard clinical complete response, in MCL.[20] Indeed, there was no difference in OS between patients in complete or partial remission at the end of treatment, as defined by the IWG criteria with or without FDG-PET. This finding supports the notion that PET and molecular MRD provide different prognostic information in MCL, probably because they are measuring different types of disease activity, in different disease compartments, with differing sensitivities. The maximum standardized uptake value (SUVmax) defined by FDG-PET, also described as an independent prognosis factor, was not analyzed in our cohort.[27]

Unexpectedly, neither the MIPI nor Ki67 scores (30% cut-off) had any impact on PFS or OS with the RiBVD regimen. This may reflect differences in treatment efficacy by RiBVD in patients with high-risk MIPI scores (70% OS at 36 months), compared to the efficacy of historical treatment controls in the original patient cohort that was used to define the high-risk MIPI score (40% OS at 36 months).[28]

Peripheral blood, but not bone marrow-based MRD status, was highly predictive of PFS and OS in this study (4-year OS of 86.6% for MRD-negative patients com-

pared to 28.6% for MRD-positive patients; (P<0.0001). While for peripheral blood this is broadly in keeping with findings of the EU-MCL network for other treatment regimens,[20] results concerning the prognostic impact of bone marrow molecular MRD, in patients treated with RiBVD, differ.[20] The prognostic impact of MRD in patients treated with the R-BAC500 regimen has not been reported as yet.[24] One avenue of investigation for clarification of these issues will be testing of 'next generation' cellular and molecular methods of MRD detection. Multi-parametric flow cytometry, although requiring very high levels of expertise, has been shown to be feasible and provide satisfactory sensitivity, when compared to highly standardized quantitative polymerase chain reaction methods in MCL.[29] For molecular MRD, droplet digital polymerase chain reaction analysis is gaining interest[30] as is molecular MRD assessment in circulating cell-free DNA in B-cell non-Hodgkin lymphoma.[31-33] Combinatorial approaches (metabolic, cellular/molecular) as reported here for MCL, and in follicular lymphoma,[34] will also be useful. Ultimately, careful investigation of residual disease, particularly by combinatorial approaches, will be needed to further refine MRD-driven precision medicine approaches in lymphoma, and other cancers.[35]

MRD positivity at the end of treatment or at 1 year of follow-up was found to be highly predictive for early relapse (at 11 and 26 months, respectively) in patients treated with the RiBVD regimen. Although numbers of MRD-negative patients were small, these findings add further weight to the notion that achieving durable molecular remission is an important goal in MCL. Indeed, maintenance therapy and/or pre-emptive treatment directed to patients in molecular relapse or remaining MRD-positive after treatment has been shown to play a significant role in prolonging clinical response in MCL.[2,23,36,37] The choice of maintenance or a pre-emptive therapy strategy may depend on the nature of initial therapy, as highlighted by a recent study that failed to show the benefit of rituximab maintenance after bendamustine.[38]

In conclusion, our results identify the combination of rituximab, bendamustine bortezomib and dexamethasone, without maintenance therapy, as a promising treatment option in MCL patients ≥65 years old. The RiBVD regimen compares favorably with other treatment strategies used in this setting, although randomized trials are still lacking. Prolonged PFS appears to result from rapid clearance of (re)circulating tumor B cells in the post-induction phase. Continued molecular remission in the blood was predictive of prolonged survival, indicating that molecular MRD monitoring and molecular response offer significant potential as precision medicine tools for early and late clinical decision-making in MCL.

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