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LETTER TO THE EDITOR

Molecular remission is an independent predictor of progression-free survival in patients with Waldenström macroglobulinemia treated with chemo-immunotherapy: Results from the FIL_BIOWM study

To the Editor,

Waldenström macroglobulinemia (WM) is a mature B-cell neoplasm characterized by bone marrow (BM) infiltration by lymphoplasmacytic lymphoma and a monoclonal IgM protein in the serum.[1] The past 2 decades have witnessed important treatment advances, with the introduction of chemo-immunotherapy (CIT) in the early 2000s and ibrutinib in more recent years. Despite these progresses, most patients eventually relapse after treatment. The depth of clinical response following rituximab-based therapy has revealed an important predictor of progression-free survival (PFS).[2]

The MYD88 (L265P) somatic mutation is detectable in approximately 95% of WM patients[3] whereas alternative MYD88 mutations are rare.[4] The high prevalence of a single somatic mutation makes MYD88 (L265P) a suitable marker for the assessment of molecular response after treatment. While minimal residual disease (MRD) has a well-established prognostic role in other mature B-cell neoplasms such as follicular lymphoma[5] or mantle cell lymphoma,[6,7] its role in WM is not yet defined. The FIL_BIOWM study (NCT03521596) is a multicenter retrospective and prospective observational study, including 300 patients with either IgM monoclonal gammopathy of undetermined significance (IgM-MGUS) or WM. One of the aims of the study is the assessment of MRD after treatment and its correlation with long-term outcomes in WM patients. In this study we analyzed the retrospective series of WM patients treated with CIT with the aim to evaluate the rate and prognostic impact of achieving a molecular remission.

We focused on the retrospective series of the FIL_BIOWM study and analyzed WM patients who met all the following eligibility criteria: diagnosis of WM according to International Workshop on Waldenström Macroglobulinemia (IWWM) criteria [1]; presence of MYD88 (L265P) mutation; need of treatment according to IWWM recommendations [8]; treatment with CIT; availability of both baseline and post-treatment BM samples. The study was conducted in accordance with the principles of Declaration of Helsinki and approved by ethics committees of participating institutions. The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000, and subjects provided written informed consent. Clinical response after treatment was assessed according to the VI IWWM criteria.[9]

The allelic frequency of MYD88 (L265P) mutation was assessed in CD19+ BM mononuclear cells (MNC) collected after treatment using allele specific real-time quantitative polymerase chain reaction (AS-qPCR) as previously described.[4] Cell lines OCI-LY19 (MYD88 wt) and OCI-LY3 (MYD88 mutated, L265P) were used to construct two different standard curves by dilution series of 7 different concentrations ranging from 40 ng/μl to 0.08 ng/μl corresponding to allele burdens ranging from 100% to 0.5%. Molecular remission was defined as undetectable MRD (uMRD) after treatment.

Qualitative variables were described as counts and percentage. Quantitative variables were summarized as median and Interquartile Range (IQR). The association between two categorical variables was tested via Fisher's exact test. The agreement between clinical and molecular response was evaluated using the Cohen's Kappa coefficient.

Overall Survival (OS) was defined as the time between diagnosis and death or last follow-up, while PFS was calculated as the time from entry onto this study until lymphoma progression or death as a result of any cause.[10] OS and PFS were estimated by Kaplan-Meier product limit method and log-rank test was used to compare PFS between groups. A predefined multivariable proportional hazard Cox model was used to adjust the prognostic effect of MRD for the effect of confounders. *p*-values lower than 0.05 were considered significant. All statistical analyses were performed using Stata 17 (StataCorp. 2021. *Stata Statistical Software: Release 17*. College Station, TX: StataCorp LLC).

The characteristics of the 54 eligible patients are shown in Table 1. The overall response rate (ORR) was 92.6%. Twenty-one patients (38.9%) had uMRD after therapy and 14/21 (66.7%) of them were in CR or VGPR. Among 24 patients achieving CR/VGPR, 14 (58%) had uMRD, whereas among 26 patients who obtained PR/MR, 7 had (27%) uMRD after therapy, with a fair agreement between clinical and molecular response (Cohen's Kappa 0.35, 95% confidence interval 0.10–0.61).

The median allelic frequency of MYD88 (L265P) before therapy was 32.2 (IQR 4.9–45.9) and was similar in patients who achieved uMRD as compared with those with detectable MRD after treatment ($p = 0.39$). The median allelic frequency after therapy was 0.2 (IQR:

TABLE 1 Patients' baseline characteristics

Characteristic	
Age (years), median (IQR)	65 (55–70)
Gender, *n.* of patients (%)	
Female	18 (33%)
Male	36 (67%)
ISSWM, *n.* of patients (%)	
Low	11 (27.5%)
Intermediate	14 (35%)
High	15 (37.5%)
Line of therapy, *n* of patients (%)	
1	47 (87%)
2	4 (7%)
≥3	3 (6%)
Chemo-immunotherapy, *n* of patients (%)	
Rituximab-Bendamustine	28 (52%)
FCR	3 (6%)
DRC	18 (33%)
R-CVP	3 (6%)
R-CHOP	2 (4%)
Response, *n* of patients (%)	
Complete remission	5 (9.3%)
Very good partial response	19 (35.2%)
Partial response	25 (46.3%)
Minimal response	1 (1.8%)
Stable disease	4 (7.4%)

Abbreviations: FCR, Fludarabine, Cyclophosphamide, Rituximab; DRC, Desamethasone, Rituximab, Cyclophosphamide; ISSWM, International Scoring System for Waldenström macroglobulinemia; R-CVP, Rituximab, Cyclophosphamide, Vincristine, Prednisone; R-CHOP, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone.

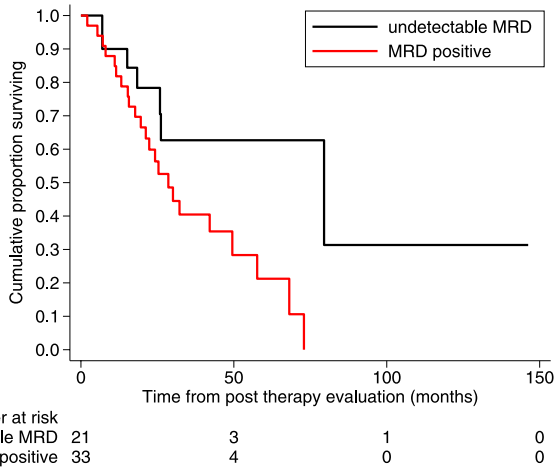

FIGURE 1 Progression-free survival according to minimal residual disease (MRD) status at the end of therapy

0–1.6) corresponding to a median percentage reduction of 99% (IQR: 91%–100%) as compared with baseline values.

The rate of molecular responses was not significantly different following nucleoside analogs (41.9%) and alkylating-based regimens (34.8%) ($p = 0.778$), as it happened with clinical response. The median decrease of MYD88 allelic frequency was respectively 99.7% (IQR 94%–100%) and 97.6% (IQR 64.4%–100%) ($p = 0.114$), albeit the decrease was faster in the former group (median slope −0.14, IQR −0.18; −0.04 vs. median slope −0.02, IQR −0.1; −0.003) ($p = 0.001$).

With a median follow-up from the start of therapy of 41 months (IQR: 28–63), 23/54 patients (42.6%) have progressed and 12/54 (22.2%) have died. The 5-year OS was 75.3% (95% CI: 58.3%–86.2%). The PFS was significantly longer for patients with uMRD as compared with patients with detectable MRD at the end of therapy (median PFS: 79.5 vs. 28.6 months respectively, $p = 0.030$) (Figure 1). After adjusting for type and line of therapy in a predefined multivariable model, uMRD still retained its association with PFS, with a worse prognosis for patients not achieving a molecular response after treatment (HR = 2.77 $p = 0.034$) (Table 2).

In the last 2 decades CIT has represented the standard treatment for WM. Rituximab in combination with Bendamustine or with Dexamethasone and Cyclophosphamide (DRC) are the most widely used regimens and are both highly effective, though some retrospective data suggest a better PFS in patients receiving Rituximab-Bendamustine as compared with DRC.[11,12] In this study we confirmed the efficacy of both nucleoside-based or alkylating-based regimens. The high rate of VGPR and CR rate likely reflects the higher propensity to repeat BM assessment at the end of therapy in patients attaining a deep clinical response, as compared with patients with clinical PR or MR. While it has been demonstrated that the depth of clinical response is a predictor of PFS,[2] the prognostic impact of MRD in WM has been only marginally evaluated by flow cytometry in two studies where achieving less than 5% residual monoclonal B-cells was associated with a better outcome, although best clinical responses and longer PFS could only be achieved when the residual cells were cleared below the limit of detection (10^{-3}-10^{-4}).[13,14] The value of achieving a molecular response with CIT has never been investigated in WM. Here we demonstrated for the first time that molecular remission is an independent predictor of PFS. The retrospective nature and the small sample size are intrinsic limitations, whereas the selection of patients homogeneously treated with fixed duration CIT, mostly frontline, represents the strength of this study. We acknowledge that the sensitivity of AS-qPCR is relatively low and that with a more sensitive method the rate of molecular response would be lower than in our study. Actually, we aim to validate these results in the prospective cohort of the FIL_BIOWM study using both AS-PCR and digital PCR (dPCR). At diagnosis, the MYD88 (L265P) mutation is detectable by dPCR with similar rates and allele frequencies in plasma and BM samples.[15] If confirmed in post-treatment samples, dPCR on cell-free DNA from plasma could become the ideal method to monitor MRD in WM patients.

TABLE 2 Effect of undetectable MRD after therapy on progression-free survival, adjusted for type and line of therapy (multivariable Cox regression model)

	Hazard ratio	95% confidence interval	*p* Value
MRD status after therapy (detectable vs. undetectable)	2.77	1.08–7.09	0.034
Type of therapy (alkylating-based regimen versus nucleoside analogs-based therapy)	0.87	0.4–1.87	0.717
Line of therapy (≥2 vs. 1)	0.9	0.3–2.66	0.845

AUTHOR CONTRIBUTIONS

Marzia Varettoni designed the study, analyzed data and wrote the manuscript; Nicole Fabbri, Irene Dogliotti, Emilia Cappello, Chiara Cavalloni, Giulia Vittoria Facchetti, Martina Ferrante participated in the collection and analysis of data; Virginia Valeria Ferretti performed statistical analysis; Silvia Zibellini, Daniela Drandi, Daniela Furlan, Cristina Jiménez and Chiara Varraso performed molecular analyses; Simone Ferrero, Michele Merli, Luca Arcaini and Ramon Garcia-SanzsS critically revised the manuscript.

KEYWORDS

minimal residual disease, MYD88, progression-free survival, Waldenström Macroglobulinemia

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CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

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