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by Emilia Cappello, Marco Lucioni, Professor, Emanuela Boveri, Virginia V. Ferretti, Gianmarco Favrin, Cristina Picone, Silvia Zibellini, Sara Rattotti, Chiara Varraso, Martina La Fauci, Giulia V. Facchetti, Chiara Cavalloni, Beatrice Cuniolo, Marco Paulli, Luca Arcaini and Marzia Varettoni

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Prognostic significance of monotypic plasma cell infiltration of the bone marrow in patients with Waldenström macroglobulinemia

Running head: Prognostic impact of plasma cells in Waldenström macroglobulinemia

Emilia Cappello[1], Marco Lucioni [2,3], Emanuela Boveri[3], Virginia V. Ferretti[4], Gianmarco Favrin[2], Cristina Picone[5], Silvia Zibellini[5], Sara Rattotti[1], Chiara Varraso[1], Martina La Fauci[1], Giulia V. Facchetti[2], Chiara Cavalloni[1], Beatrice Cuniolo[1], Marco Paulli[2,3], Luca Arcaini[1,2], Marzia Varettoni[1]

(1) SC Ematologia 1, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
(2) Department of Molecular Medicine, University of Pavia, Italy
(3) Unit of Anatomic Pathology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
(4) SSD Biostatistica e Clinical Trial Center, Fondazione I.R.C.C.S. Policlinico San Matteo, Pavia, Italy
(5) Unit of Molecular Hematology and Precision Medicine, Fondazione IRCCS Policlinico San Matteo Pavia, Italy

Corresponding author
Marzia Varettoni, MD
SC Ematologia 1, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy
Phone: +39-0382-503076
e-mail: m.varettoni@smatteo.pv.it

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MV: received honoraria from AbbVie, AstraZeneca, Beone, Johnson &Johnson, Lilly, received research support from Beone and served on advisory board for AstraZeneca and Lilly.
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Author contributions
- M.V. designed the research study;
- M.V. and E.C. interpreted the data and wrote the paper;
- M.V., E.C., G.F., S.R., M.L.F, G.V.F., C.C., B.C. collected clinical data;
- C.V., S.Z. and C.P. collected biological data and performed laboratory testing;
- M.L., E.B. and M.P. performed the histological revision;
- V.V.F. performed statistical analysis;
- E.C., G.F., S.R., C.C., G.V.F., L.A. and M.V. critically revised the manuscript;
- All the authors revised and approved the final version of the manuscript.

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Main text

To the Editor,

Waldenström Macroglobulinemia (WM) is a rare, indolent B cell lymphoma, corresponding to a lymphoplasmacytic lymphoma associated with a serum monoclonal immunoglobulin M (IgM) paraprotein.[1] The bone marrow (BM) neoplastic infiltrate typically consists of small lymphocytes, plasma cells (PCs) and cells with intermediate features, known as plasmacytoid lymphocytes.[2] According to current diagnostic[3] and response criteria[4], a trephine BM biopsy with an adequate immunohistochemistry (ICH) is required to confirm both the diagnosis and a complete response after treatment in WM patients. Over the past 20 years, chemoimmunotherapy (CIT) has represented the mainstay of WM treatment, with overall response rates (ORRs) exceeding 80% with the most widely used regimens, although Bruton's tyrosine kinase inhibitors (BTKi) have more recently emerged as an established standard therapeutic option. Despite high chemosensitivity, persistence of monotypic PCs after CIT in absence of demonstrable monotypic B lymphocytes has been reported.[5] Currently, the prognostic value of residual monotypic PCs infiltration after chemoimmunotherapy remains unclear. In this study, we assessed PCs infiltration in the BM biopsies of patients with symptomatic WM treated with CIT with the aim to correlate PCs infiltration with outcome.

We retrospectively reviewed WM patients (pts) consecutively diagnosed and treated with CIT at the Division of Hematology of Fondazione IRCCS Policlinico San Matteo, Pavia (Italy) between June 2005 to September 2023. For the purposes of this study, the extent of BM infiltration of B-lymphocytes and PCs was assessed by hematopathologists using ICH using with B-cell markers (CD20, PAX5, CD79a) and plasmacytic differentiation markers (MUM1, CD38, CD138) respectively; PCs percentage was calculated as a proportion of the total lymphoplasmacytic lymphoma (LPL) cluster rather than as a percentage of the total BM cellularity. Clonality for both B-cells and PCs was assessed via light-chain restriction in pre- and post-therapy samples. In the post-treatment setting, 'disease persistence' was defined as the presence of >10% clonal B-cells; the presence of any level of light-chain restricted PCs was defined as "monotypic PCs infiltration". The *MYD88* (L265P) mutation was assessed using allele-specific quantitative PCR (AS-qPCR), while *TP53* and *CXCR4* mutations were evaluated using next-generation sequencing. Post-treatment progression free survival (PFS) was defined as the time from BM biopsy after CIT to either disease progression or death, whichever occurred first, or censored at last follow-up. Post-treatment PFS was estimated via Kaplan-Meier method. No baseline exclusion criteria were applied, however patients lacking post-treatment BM biopsy and residual PCs clonality were excluded from the post-treatment PFS analysis. Statistical analyses were performed using Stata 18 software. This study did not require formal ethics committee approval, but it complied with all relevant national ethical standards.

The study population consisted of 92 WM patients with a median age at diagnosis of 65.7 (interquartile range (IQR): 58.2-72.2). Patients' characteristics are summarized in (Table 1). The *MYD88* (L265P) mutation was detected in 82/90 pts (91%), 37% of patients also harbored a *CXCR4* mutation. *TP53* mutational status was available in 62 patients and 4 (6.4%) had a *TP53* mutation. The IPSS-WM score[6] was evaluable for 90 out of 92 patients, classifying 18.9%, 46.7%, and 34.4% of them into low-, intermediate-, and high-risk categories, respectively; detailed risk stratifications according to the revised IPSS-WM (rIPSS-WM)[7] and Modified Staging System for WM (MSS-WM)[8] models are reported in (Table 1). Before treatment, the median IgM paraprotein level and serum β_2-microglobulin were 2730 mg/dL and 3611 mcg/L respectively. In the bone

marrow biopsy performed before starting therapy, the median B-cell infiltration was 40% (IQR: 30-70) and median PCs infiltration was 10% (IQR: 7.5-15). We first investigated if there were any clinical and biological differences in patients with a PCs infiltrate ≥10% in baseline BM biopsy. Patients with ≥10% PCs at baseline had significantly higher IgM levels (median 3540 mg/dL, IQR: 1660-5510) as compared with those with <10% PCs (median 962 mg/dL, IQR: 833-2388) (p=0.002). In addition, we found that *CXCR4* mutations were more common in patients with 10% or more PCs as compared with those having <10% PCs before therapy (45% versus 15%, p=0.028), whereas there was no difference in the prevalence of *MYD88* (L265P) (p=0.192) or *TP53* mutations (p=0.244). The median time from diagnosis to CIT was 5.7 months (IQR: 1.4-37.7). Eighty-three patients (90.2%) received CIT as first-line therapy, and 9 (9.8%) as second-line treatment (7 after chlorambucil). In detail, 54 patients (59%) received Rituximab-Bendamustine (BR), 32 (35%) Rituximab-Cyclophosphamide-Prednisone (R-CP), 4 (4%) Fludarabine-Cyclophosphamide-Rituximab (FCR) and 2 (2%) Rituximab+Cyclophosphamide-Doxorubicine-Vincristine-Prednisone (R-CHOP). We grouped “BR” and “FCR” patients into the “R-Purine analogues” group, while “R-CP” and “R-CHOP” were categorized as “R-Alk/R-CHOP” group. The ORR according to IWWM-11 response criteria[4] was 91%, including 8.7% complete responses (CR), 30.4% very good partial responses (VGPR), 45.7% partial responses (PR) and 6.5% minor responses (MR). The ORR was similar in patients treated with R-Purine analogues as compared with those treated with R-Alk/R-CHOP (ORR 94.8% versus 85.3%, p=0.140), with a significantly higher major response rate (MRR) in the former group (MRR 91.4% versus 73.5%, p=0.034). Post-treatment BM biopsies were available in 84 out of 92 patients (91%) and were performed after a median time of 1.9 months after the last cycle of CIT (IQR: 1.4-2.3). After treatment, the median B-cell infiltration was 5% (IQR: 2-10%) and the median PCs infiltration was 5% (IQR 5-15%). An unsatisfactory response to CIT was observed in 8 patients (7 stable disease and 1 PD), showing in those patients a median post-treatment B-cell infiltration of 5% (IQR: 2–10%) and a median PC infiltration of 5% (IQR: 5–13%). These values were comparable to those observed in patients achieving a major response (CR, VGPR, or PR), where median B-cell and PCs residual infiltrations were 5% (IQR: 2–10%) and 5% (IQR: 5–15%), respectively. Based on residual B lymphocytes and PCs in BM biopsy after treatment, we grouped patients as follows: Group 1 (49 patients, 59%) with ≤10% B CD20+ lymphocytes without monotypic PCs, Group 2 (19 patients, 23%) with ≤10% B CD20+ lymphocytes and persistence of monotypic PCs, and Group 3 (15 patients, 18%) with >10% residual B CD20+ lymphocytes. With a median follow-up of 6.3 years (IQR: 3.3-9.6) from the time of diagnosis and of 4.4 years (IQR: 1.7-6.8) from the end of treatment, the median post-treatment PFS was similar across the three groups (p=0.209) (Figure 1).

In this study we evaluated the prognostic significance of the degree of BM PCs infiltration after treatment. The main limitation of this study is the retrospective design, on the other hand the inclusion of consecutive WM patients homogeneously treated with CIT in a single institution represents a strength. Our findings align with recent data from the R2W trial[9], confirming that a failure to achieve bone marrow B-cell depletion after CIT predicts inferior PFS. Notably, this correlation remains consistent despite different methodological approaches (flow cytometry in the R2W trial and IHC in our study). However, while B-cell persistence is an established driver of poor outcome, the prognostic impact of residual plasma cells in WM, both at diagnosis and after treatment, remains poorly understood. The degree of PCs infiltration in BM has been associated with IgM paraprotein concentration at diagnosis in WM.[10] In addition, Zanwar et al[11] demonstrated that an excess of PCs at diagnosis was associated with a trend towards shorter time to next treatment and overall survival. In our study, we found that WM pts with ≥10% PCs at diagnosis were more

frequently *CXCR4*-mutated and had higher IgM levels. As previously reported, *CXCR4*-mutated patients are characterized by increased BM disease involvement, high serum IgM levels, symptomatic hyperviscosity at diagnosis and shorter time to first treatment.[12] After treatment with CIT, nearly a quarter of patients in our study showed persistence of monotypic PCs in absence of clonal lymphocytes. The finding of residual PCs after treatment has been previously reported[5,13] and may account for the persistence of IgM paraproteinemia after CIT. A late clearance of these PCs, which do not express CD20 and are then more resistant to CIT, may explain the deepening of response over time observed in some studies, especially in patients treated with purine analogues. Varghese et al[13] reported a median time of 6 months (range: 0-18) to achieve maximum response in a cohort of 16 pts treated with Fludarabine or Fludarabine plus Cyclophosphamide. Similarly, Del Giudice and colleagues[14] found that in more than 50% of patients the maximum response to therapy was achieved at median time of 8 months (range: 3-35) from the start of therapy in 13 WM/LPL pts treated with fludarabine-containing regimes. Actually, in our study response assessment was performed after a median time of 1.9 months after the end of treatment, leading to a potential underestimation of response that could be overcome postponing disease assessment after CIT. A late clearance of PCs may explain why the persistence of monotypic PCs at the end of CIT was not associated with earlier disease progression after CIT in our study, and suggests that there is no need of additional consolidation treatment aimed at eliminating residual PCs. In conclusion, understanding the prognostic significance of monotypic PCs bone marrow infiltration remains an area that warrants further investigation in WM.

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Table

Table 1. Patient characteristics of the WM cohort (n=92).

All patients (n=92)	
Clinical variables	(n=92), [missing]
Age, (years)	
Median, (interquartile range [IQR])	65.7 (58.2-72.2)
Sex	
Male, n (%)	52 (56%)
Female, n (%)	40 (44%)
IPSSWM score[6]	
Low, n (%)	17 (18.9%) [2]
Intermediate, n (%)	42 (46.7%) [2]
High, n (%)	31 (34.4%) [2]
rIPSS-WM score[7]	
Very low, n (%)	16 (20%) [12]
Low, n (%)	24 (30%) [12]
Intermediate, n (%)	18 (22.5%) [12]
High, n (%)	14 (17.5%) [12]
Very high, n (%)	8 (10%) [12]
MSS-WM score[8]	
Low, n (%)	26 (31%) [8]
Low-intermediate, n (%)	24 (28.6%) [8]
Intermediate, n (%)	18 (21.4%) [8]
High, n (%)	16 (19%) [8]
Molecular variables	
***MYD88*^{L265P}-MUT**	
Present, n (%)	84 (91%) [2]
***CXCR4*-MUT**	
Present, n (%)	34 (37%) [19]
TP53-MUT	
Present, n (%)	4 (6%) [30]
Biological variables (pre-treatment)	
Hemoglobin, (g/dl)	
Median, (IQR)	10.2 (9.0-11.9) [1]
Platelets, (x10^9/L)	
Median, (IQR)	260.0 (170.0-345.0) [1]
Serum albumin, (g/dl)	
Median, (IQR)	3.6 (3.3-4.0) [3]
Serum B2-microglobulin, (mcg/L)	
Median, (IQR)	3611 (2882.0-5500.0) [9]
LDH, (U/L)	
Median, (IQR)	161 (131-211) [7]
IgM paraprotein, (g/dl)	
Median, (IQR)	2.3 (1.2-3.6) [1]
Serum IgM level, (mg/dl)	
Median, (IQR)	2730 (1260-4740) [39]
Histological variables (pre-treatment)	
B-lymphocytes infiltration at BM biopsy, (%)	
Median, (IQR)	40 (30-70)
PCs infiltration at BM biopsy, (%)	
Median, (IQR)	10 (7.5-15)
PCs (%) at BM biopsy	
<10%, n (%)	23 (25%)
≥10%, n (%)	69 (75%)
Treatment features	
Type of CIT	
R-Alk/R-CHOP group°, n (%)	34 (37%)

R-Purine analogues group[#], n (%)	58 (63%)
Line of therapy	
1, n (%)	83 (90.2%)
2, n (%)	9 (9.8%)
Response (as per IWWM11 response criteria)[4]	
CR, n (%)	8 (8.7%)
VGPR, n (%)	28 (30.4%)
PR, n (%)	42 (45.7%)
MR, n (%)	6 (6.5%)
SD, n (%)	7 (7.6%)
PD, n (%)	1 (1.1%)
Post-treatment BM biopsy	
Yes, n (%)	84, (91%)
B-lymphocytes infiltration at post-treatment BM biopsy (%)	
Median, (IQR)	5 (2-10), [8]
PCs infiltration at post-treatment BM biopsy (%)	
Median, (IQR)	5 (5-15), [8]
Patients' groups according to residual B-lymphocytes and PCs in BM biopsy after treatment	
Group 1, n (%)	49 (59%)
Group 2, n (%)	19 (23%)
Group 3, n (%)	15 (18%)

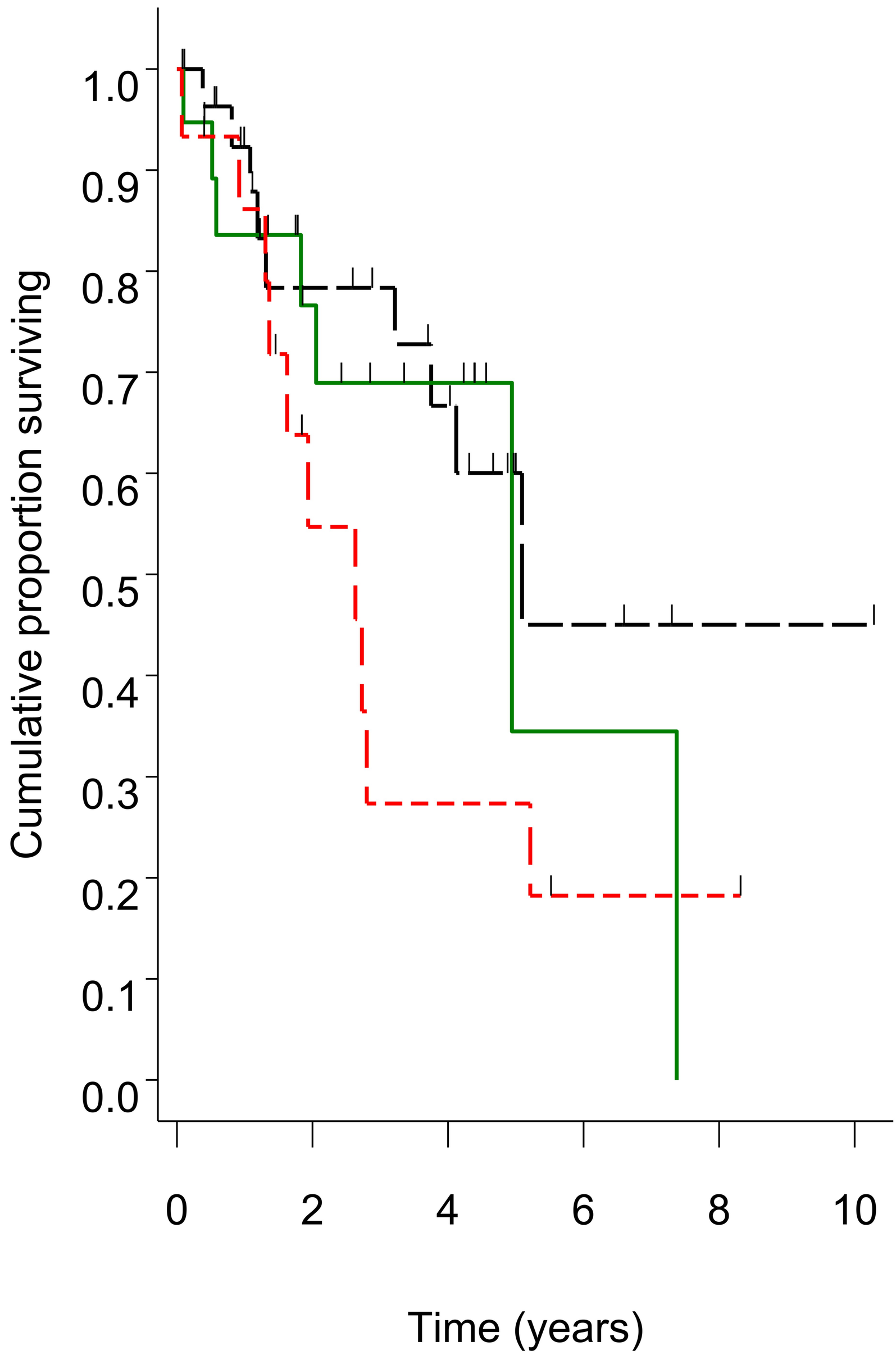
BM= Bone marrow. CIT= Chemoimmunotherapy. CR= Complete Response. IPSSWM = International prognostic scoring system on Waldenström macroglobulinemia. IgM= Immunoglobulin M. IQR = Interquartile range; LDH = Lactate dehydrogenase. PCs= Plasma cells. MR= Minor response. MSS-WM: Modified Staging System for WM. PR= Partial response. PD= Progressive disease. SD= Stable disease. rIPSS-WM= revised IPSS-WM. VGPR= Very good partial response. WM= Waldenström macroglobulinemia.

°R-Alk/R-CHOP group: R-CP (Rituximab-Cyclophosphamide-Prednisone) and R-CHOP (Rituximab+Cyclophosphamide-Doxorubicine-Vincristine-Prednisone). [#]R-purine analogues: FCR (Fludarabine-Cyclophosphamide-Rituximab) and BR (Rituximab-Bendamustine).

Group 1: ≤10% B CD20+ lymphocytes without monotypic PCs; Group 2: ≤10% B CD20+ lymphocytes and persistence of monotypic PCs; Group 3: >10% residual B CD20+ lymphocytes.

Figure legend

Figure 1. Post-treatment PFS according to B CD20+ lymphocytic infiltration and residual monotypic PCs in post-treatment bone marrow biopsy [PFS=Progression Free Survival, PCs=Plasma cells]. Group 1 **(green curve):** B CD20+ lymphocytes ≤10% without monotypic PCs (n=49, 59%); Group 2 **(black curve):** B CD20+ lymphocytes ≤10% with persistence of monotypic PCs (n=19, 23%); Group 3 **(red curve):** B CD20+ lymphocytes >10% (n=15, 18%).



Number at risk

	0	2	4	6	8	10
Group 1 (green solid curve)	19	10	6	1	0	0
Group 2 (black long dash curve)	29	16	11	3	1	1
Group 3 (red short dash curve)	15	6	3	1	1	0