Deepening of response after completing rituximab-containing therapy in patients with Waldenstrom macroglobulinemia

Jorge J. Castillo1,2 | Joshua N. Gustine1,3 | Andrew Keezer1 | Kirsten Meid1 | Catherine A. Flynn1 | Toni E. Dubeau1 | Gloria Chan1 | Jiaji Chen1 | Maria G. Demos1 | Maria L. Guerrero1 | Cristina Jimenez1 | Amanda Kofides1 | Xia Liu1 | Manit Munshi1 | Nicholas Tsakmaklis1 | Christopher J. Patterson1 | Lian Xu1 | Guang Yang1 | Zachary R. Hunter1 | Steven P. Treon1,2

1Bing Center for Waldenström Macroglobulinemia, Dana-Farber Cancer Institute, Boston, Massachusetts
2Department of Medicine, Harvard Medical School, Boston, Massachusetts
3Department of Medicine, Boston University School of Medicine, Boston, Massachusetts

Correspondence
Jorge J. Castillo, 450 Brookline Ave, Mayer 221, Boston, MA 02215.
Email: jorgej_castillo@dfci.harvard.edu

Funding information
American Society of Hematology

Abstract
Rituximab-containing regimens are commonly used for frontline therapy in patients with symptomatic Waldenström macroglobulinemia (WM). We had observed that a portion of WM patients experienced deepening of response months to years after therapy completion. We carried a retrospective study aimed at describing this phenomenon. We gathered baseline data, and responses at end of induction, end of maintenance and best response. Deepening of response was defined as ≥25% decrease in serum IgM achieved at a later time from therapy completion. Of 178 patients included, 116 (65%) received maintenance therapy and 62 (35%) were observed. In patients who received maintenance, 44 (38%) had ≥25% decrease in serum IgM level after the end of maintenance with a median time from end of maintenance to lowest IgM level of 1.6 years (range 0.1-7.9 years). In patients who were observed, 19 (31%) had ≥25% decrease in serum IgM level after the end of induction with a median time from end of induction to lowest IgM level of 1.6 years (range 0.2-5.1 years). Baseline hemoglobin <11.5 g/dL, bone marrow involvement ≥50%, CXCR4 mutations and serum IgM ≥4000 mg/dL were associated with lower odds of deepening of response after therapy completion. Deepening of response was associated with better progression-free survival (PFS; HR 0.46, 95% CI 0.26-0.80; P = .006) and better survival after frontline treatment initiation (SAFTI; HR 0.21, 95% CI 0.06-0.73; P = .01). In conclusion, deepening of response occurs in one third of WM patients after completing rituximab-containing regimens and was associated with better PFS and SAFTI.

1 | INTRODUCTION

Waldenstrom macroglobulinemia (WM) is a rare and incurable lymphoma characterized by the uncontrolled accumulation of IgM-secreting lymphoplasmacytic cells in the bone marrow and other organs.1 Rituximab-containing regimens, such as bendamustine and rituximab (Benda-R), bortezomib, dexamethasone and rituximab (BDR) and cyclophosphamide, dexamethasone and rituximab (CDR), are commonly used for primary therapy in patients with symptomatic WM. These regimens are highly effective with overall response rates (ORR) of 80%-90% and median progression-free survival (PFS) approximating 5 years.2,3 In the absence of prospective data, maintenance rituximab can be used after completing induction therapy in WM, as it has shown to positively impact response and PFS in retrospective studies.2,4,5
Response to therapy in patients with WM is assessed by a decrease in serum IgM levels. We observed some WM patients had continual decreases in serum IgM levels months to years after completion of primary therapy with rituximab-containing regimens followed by observation or maintenance rituximab. Although this phenomenon has been previously reported in prospective studies in WM patients treated with rituximab monotherapy, the prevalence of and the factors associated with deepening of response after completion of therapy as well as its impact on survival outcomes have not been systematically evaluated previously in WM patients receiving chemoimmunotherapy.

We carried a retrospective cohort study aimed at describing the frequency of deepening of response after completion of therapy in patients with WM and at identifying predictive factors for this phenomenon. We also evaluated the potential prognostic value of deepening of response after completion of therapy in PFS and survival after frontline treatment initiation (SAFTI).

2 | METHODS

2.1 | Patient selection

We selected consecutive WM patients from a prospectively maintained database who received rituximab-containing combination therapy at our institution between January 2005 (date of database initiation) and December 2016. All patients were ≥18 years, met clinico-pathological criteria for a diagnosis of WM, as per the second International Workshop for WM (IWWM) guidelines, met criteria for treatment initiation as per the second IWWM, signed consent for therapy initiation and signed consent for having their medical records reviewed for research. This study was approved by the Institutional Review Board at Dana-Farber Cancer Institute.

2.2 | Data gathering

Pertinent data was gathered retrospectively and included clinical characteristics at therapy initiation, MYD88 and CXCR4 mutational status, responses at the end of induction, at the end of maintenance and at the lowest serum IgM level after completion of induction or maintenance, PFS and survival after first therapy initiation (SAFTI). Extramedullary disease was defined as lymphadenopathy ≥1.5 cm in diameter or splenomegaly ≥15 cm in largest axis. Bone marrow involvement was defined as the percentage of infiltration of the intertrabecular space by lymphoplasmacytic lymphoma. Responses were assessed using modified criteria from the sixth IWWM, in which extramedullary disease assessment is not mandatory for partial (PR) or very good partial response (VGPR) but is required for complete response (CR). Deepening of response was defined as ≥25% decrease in serum IgM levels after completion of primary therapy. This was determined by comparing the serum IgM level at the end of primary therapy to the serum IgM level at best response during the follow-up period between treatment completion and disease progression or death. Note, PFS was defined as the time between treatment initiation and disease progression, death or last follow-up. Also, SAFTI was defined as the time between treatment initiation and death or last follow-up. The mutational status of MYD88 and CXCR4 was assessed using allele-specific polymerase chain reaction (PCR) for MYD88 L265P and nonsense CXCR4 mutations. Frameshift CXCR4 mutations were assessed by Sanger sequencing. Manual deletion techniques for MYD88 and CXCR4 have been previously reported.

2.3 | Statistical analysis

Patients’ characteristics are presented using descriptive statistics. We assessed for differences in characteristics and response to therapy between groups using chi-square and Fisher exact tests, whenever appropriate. Univariate and multivariate logistic regression models were fitted to evaluate the association between variables and deepening of response. Outcomes are reported using odds ratio (OR) with 95% confidence interval (CI). The PFS and SAFTI rates were estimated using the Kaplan-Meier method for incomplete observations, and the survival distributions were compared using the log-rank test. Univariate and multivariate Cox proportional-hazard regression models were fitted to evaluate the association between variables and survival outcomes (ie, PFS and SAFTI). Outcomes are reported using hazard ratio (HR) with 95% CI. P values <.05 were considered statistically significant. Calculations were obtained using STATA 15 (StataCorp, College Station, TX, USA).

3 | RESULTS

3.1 | Patient characteristics

Of 178 patients who were included in our study, 56 (31%) received Benda-R, 86 (48%) received BDR, and 36 (20%) received CDR as induction therapy. After completing induction therapy, 116 (65%) received maintenance therapy and 62 (35%) were observed. Before treatment initiation, there were no differences in age, sex, time to primary therapy, primary therapy regimen, hemoglobin, platelets, β2-microglobulin, bone marrow involvement, presence of MYD88 and CXCR4 mutations and baseline serum IgM between patients who went on to receive maintenance or were observed. Characteristics of the patients before treatment initiation are shown in Table 1.

3.2 | Serum IgM levels and response to therapy

At baseline, median serum IgM levels for patients who received maintenance or were observed were 4400 mg/dL (range 155-10 020 mg/dL) and 4445 mg/dL (range 289-8100 mg/dL), respectively (P = .37 between groups). At the end of induction, median serum IgM levels were 969 mg/dL (range 33-5940 mg/dL) and 1366 mg/dL (range 114-7108 mg/dL), respectively (P = .11 between groups). Serum IgM levels at the end of induction were statistically lower than baseline for
both groups \((P < .001\) each). At the end of maintenance, median serum IgM level was 528 mg/dL \((range 10-5410 \text{ mg/dL}) (P < .001\) compared to end of induction).

In the patients who received maintenance, the median best serum IgM level was 384 mg/dL \((range 9-4759 \text{ mg/dL}) (P < .001\) compared with end of maintenance). The median time from end of maintenance to lowest IgM level was 1.6 years \((range 0.1-7.9\) years). The rate of VGPR or better increased from 14% at the end of induction to 34% at the end of maintenance, to 45% at best response. And, the rate of partial response or better increased from 72% at the end of induction to 90% at best response (Figure 1A). In the patients who were observed, the median best serum IgM level was 1253 mg/dL \((range 11-7108 \text{ mg/dL}) (P = .01\) compared with end of induction), and the median time from end of induction to lowest IgM level was 1.6 years \((range 0.2-5.1\) years). The rate of VGPR or better increased from 11% at the end of induction to 21% at best response, and the rate of PR or better increased from 61% to 68% (Figure 1B).

### 3.3 Regression analysis for deepening of response

Based on our pre-specified criteria, 63 patients \((35\%)\) experienced deepening of response after completion of therapy. Deepening of

| TABLE 1 | baseline characteristics of 178 patients with Waldenström macroglobulinemia (WM) treated with rituximab-containing regimens |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Characteristic                  | Observation \((n = 62)\) | Maintenance \((n = 116)\) | \(P\) value |
| Age, y                          | 61 (30-86)                    | 62 (33-87)                    | .58                     |
| Age ≥65 y                       | 22 (35%)                      | 46 (40%)                      | .59                     |
| Male sex                        | 39 (63%)                      | 67 (58%)                      | .53                     |
| Time to first treatment, y      | 0.6 (0.2-1.7)                 | 0.5 (0.3-0.8)                 | .31                     |
| Hemoglobin, g/dL                | 10.9 (6-17.1)                 | 10.6 (4-15.3)                 | .94                     |
| Hemoglobin ≤11.5 g/dL           | 47 (77%)                      | 81 (70%)                      | .31                     |
| Platelet count, K/μL            | 257 (42-485)                  | 231 (17-528)                  | .13                     |
| Platelet count ≤100 K/μL        | 3 (5%)                        | 15 (13%)                      | .10                     |
| Serum β2-microglobulin, mg/L    | 3.2 (1.5-12.5)                | 2.8 (1.3-19.2)                | .11                     |
| Serum β2-microglobulin >3 mg/L  | 34 (55%)                      | 48 (41%)                      | .10                     |
| Serum IgM level, mg/dL          | 4445 (289-8100)               | 4400 (155-10 020)             | .37                     |
| Serum IgM level ≥4000 mg/dL     | 34 (55%)                      | 63 (54%)                      | .90                     |
| Bone marrow involvement         | 40% (5%-95%)                  | 40% (3%-95%)                  | .68                     |
| Bone marrow involvement ≥50%    | 29 (48%)                      | 57 (49%)                      | .80                     |
| Extramedullary disease          | 25 (40%)                      | 33 (28%)                      | .10                     |
| IPSSWM 1                        | 17 (29%)                      | 39 (36%)                      | .34                     |
| IPSSWM 2                        | 29 (49%)                      | 40 (37%)                      | .37                     |
| IPSSWM 3                        | 13 (22%)                      | 28 (26%)                      | .26                     |
| MYD88 mutation                  | 22/23 (96%)                   | 30/34 (88%)                   | .94                     |
| CXCR4 mutation                  | 10/23 (43%)                   | 18/34 (53%)                   | .59                     |

Regimen used

- Benda-R 21 (34%) 35 (30%) .79
- BDR 30 (48%) 56 (48%) .48
- CDR 11 (18%) 25 (22%) .22

Abbreviations: BDR, bortezomib, dexamethasone, rituximab; Benda-R, bendamustine, rituximab; CDR, cyclophosphamide, dexamethasone, rituximab; IPSSWM, International Prognostic Scoring System for Waldenström Macroglobulinemia.
response was observed in 44 patients (38%) who received maintenance, and in 19 patients (31%) who were observed after completion of induction therapy ($P = .33$ between groups).

In the univariate logistic regression analysis, hemoglobin level $< 11.5$ g/dL (OR 0.44, 95% CI 0.23-0.88; $P = .02$), bone marrow involvement $\geq 50\%$ (OR 0.49, 95% CI 0.26-0.93; $P = .03$), CXCR4 mutations (OR 0.33, 95% CI 0.13-0.87; $P = .03$) and serum IgM level $\geq 4000$ mg/dL (OR 0.53, 95% CI 0.29-0.99; $P = .045$) were associated with lower odds of response deepening after completion of therapy.

**FIGURE 2**  Progression-free survival (PFS) estimates according to having experienced deepening of response after completion of therapy in A, entire cohort, B, patients who received maintenance after induction therapy, and C, patients who were observed after induction therapy [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 3**  Survival after first line treatment initiation estimates according to having experienced deepening of response after completion of therapy in A, entire cohort, B, patients who received maintenance after induction therapy, and C, patients who were observed after induction therapy [Color figure can be viewed at wileyonlinelibrary.com]
Age, sex, platelet count, serum β2-microglobulin level, extramedullary disease, IPSSWM, MYD88 mutation, maintenance therapy and primary therapy regimen were not associated with deepening of response after therapy completion. We then fitted two multivariate models. One model included 178 observations (all patients) and included hemoglobin level ≤11.5 g/dL, bone marrow involvement ≥50% and serum IgM level ≥4000 mg/dL. In this model, bone marrow involvement ≥50% and serum IgM level ≥4000 mg/dL were independent predictors of lower odds of deepening of response after therapy completion, with OR 0.49 (95% CI 0.25-0.97; P = .04) and OR 0.48 (95% CI 0.25-0.93; P = .03), respectively. A second model included 57 observations (patients who were genotyped for MYD88 and CXCR4 mutations) and included hemoglobin level <11.5 g/dL, bone marrow involvement ≥50%, CXCR4 mutations and serum IgM level ≥4000 mg/dL. In this model, the only predictive factor of lower odds of deepening of response was the presence of CXCR4 mutations with OR 0.33 (95% CI 0.11-0.94; P = .04).

### 3.4 Survival outcomes

At the time of this report, the median follow-up time for all patients was 5.7 years (95% CI 4.9-6.8 years), 58 patients (32%) have progressed and 17 (10%) have died. The median follow-up for patients who experienced deepening of response was 6.3 years (95% CI 4.9-7 years) vs 5.5 years (95% CI 4.1-6.9 years) for patients who did not experience deepening of response following therapy (log-rank P = .48).

The median PFS for the entire group was 6.5 years (95% CI 5.5-not reached [NR]), and the 5-year PFS rate was 66% (95% CI 57%-74%). The median PFS was longer for patients who experienced deepening of response after therapy completion than for patients who did not (8.8 years, 95% CI 5.9-NR vs 5.9 years, 95% CI 4.1-NR, respectively). The 5-year PFS rate was higher for patients who experienced deepening of response than for patients who did not (78%, 95% CI 64-88% vs 59%, 95% CI 46-70%, respectively; P = .005; Figure 2A). Patients who experienced deepening of response after therapy completion had a lower risk of progression or death than patients who did not (HR 0.46, 95% CI 0.26-0.80; P = .006). Results were similar when evaluating deepening of response as a discrete time-varying covariate (HR 0.46, 95% CI 0.27-0.75; P = .003). Factors associated with worse PFS included age ≥65 (HR 1.89, 95% CI 1.02-3.41; P = .04) and treatment with CDR (vs Benda-R; HR 2.65, 95% CI 1.15-6.10; P = .02). Maintenance rituximab was associated with better PFS (HR 0.27, 95% CI 0.16-0.46; P < .001). Hemoglobin ≤11.5 g/dL, platelets ≤100 K/μL, serum β2-microglobulin ≥3 mg/L, serum IgM level ≥7000 mg/dL, bone marrow involvement ≥50%, MYD88 mutation, CXCR4 mutation and BDR (vs Benda-R) were not associated with better or worse PFS. In a multivariate model adjusting for age >65, frontline treatment and maintenance, deepening of response was independently associated with a better PFS (HR 0.51, 95% CI 0.28-0.96; P = .036). Similar results were found when fitting a multivariate model adjusting for age, frontline treatment, maintenance and deepening of response as a discrete time-varying covariate (HR 0.51, 95% CI 0.30-0.88; P = .02).

For the 116 patients who received maintenance after induction, the median PFS was 8.8 years (95% CI 6.4-NR). The median PFS was not reached for patients who experienced deepening of response after therapy completion while it was 6.5 years (95% CI 5.8-NR) for the patients who did not deepen response. The 5-year PFS rate was higher for patients who experienced deepening of response after therapy completion than for patients who did not (87%, 95% CI 71%-94% vs 68%, 95% CI 50%-80%, respectively; P = .008; Figure 2B). For the 62 patients who were observed after induction, the median PFS was 4.2 years (95% CI 2.1-5.5). The median PFS was longer for patients who experienced deepening of response after therapy completion than for the patients who did not (5.3 years, 95% CI 2.6-NR vs 2.6 years, 95% CI 1.4-NR, respectively). There was no detectable difference in 5-year PFS rate between patients who experienced deepening of response after therapy completion and patients who did not (52%, 95% CI 20%-77% vs 42%, 95% CI 23%-59%, respectively; P = 0.20; Figure 2C).

The 5-year SAFTI rate was higher for patients who experienced deepening of response than for patients who did not (100% vs 79%, 95% CI 64%-89%, respectively; P = .007; Figure 3A). Patients who experienced deepening of response after therapy completion had a lower risk of death than patients who did not (HR 0.21, 95% CI 0.06-0.73; P = .01). Similar results were obtained when evaluating deepening of response as a discrete time-varying covariate (HR 0.21, 95% CI 0.06-0.67; P = .008). Age ≥65 years, hemoglobin ≤11.5 g/dL, platelets ≤100 K/μL, serum β2-microglobulin ≥3 mg/L, serum IgM level ≥7000 mg/dL, bone marrow involvement ≥50%, MYD88 mutation, CXCR4 mutation, frontline treatment, maintenance and IPSSWM were not associated with better or worse SAFTI.

For the 116 patients who received maintenance after induction, the 5-year SAFTI rate was higher for patients who experienced deepening of response after therapy completion than for patients who did not (100% vs 79%, 95% CI 60%-90%, respectively; P = .02; Figure 3B). For the 62 patients who were observed after induction, there was no detectable difference in 5-year SAFTI rate between patients who experienced deepening of response after therapy completion and patients who did not (100% vs 80%, 95% CI 48%-93%, respectively; P = .15; Figure 3C).

### 4 Discussion

Herein, we present results from a study on a previously unreported phenomenon of deepening of response after therapy completion, in patients with WM treated with frontline rituximab-containing regimens. Deepening of response was defined as a further decrease in serum IgM level by at least 25% after completion of therapy. We chose this cutoff as a decrease by 25% in serum IgM levels represents a minor response (mR) in patients with WM, according to current response criteria, and could be considered clinically significant. Based on our results, a third of WM patients who receive primary therapy with rituximab-containing regimens experience deepening of response after completing therapy.
Our results are of clinical relevance, as we had previously reported on longer PFS in WM patients obtaining deeper responses to rituximab-containing therapy. In such study, 159 patients received therapy with rituximab-containing regimens, and the rates of CR, VGPR, PR and mR at best response were 9%, 13%, 50%, and 19%, respectively. The median PFS for patients who attained VGPR or better at best response was estimated at 72 months, while the median PFS for patients who attained mR or better was approximately 40 months (log-rank P = .03). The present study provides additional insights into deepening of response and the association of depth of response and survival outcomes in WM patients.

Similar decreases of approximately 10%-20% in 5-year PFS and SAFTI rates were observed in WM patients who experienced deepening of response after therapy completion, regardless if patients received maintenance or were observed after induction therapy. These differences in PFS and SAFTI rates were statistically significant in patients who received maintenance rituximab, but it was not statistically significant in patients who were observed after induction. The lack of significance in the latter scenario could be explained in part by the smaller sample size, which should not detract from the actual difference observed. Overall, these results show internal consistency.

Not only we observed a decrease in serum IgM levels, the absolute rate of VGPR increased by 10%, and the rate of PR or better by 7%. This was after a median time of 1.6 years after completion of induction therapy, in patients who were observed after completion of induction therapy. Similar results were found in patients who underwent induction and maintenance therapy, with absolute increases of 10% and 7% in VGPR and PR or better after completion of maintenance therapy, with a median time to best response of 1.6 years. It was also interesting to see that, in a small group of patients, the lowest serum IgM level was not reached until 7 years after therapy completion. This finding is of interest, especially for those patients affected by the toxic effects of IgM (eg, neuropathy, cryoglobulins and cold agglutinins), in whom a deeper response would be highly desirable to further prevent IgM-mediated tissue damage.

The reasons behind the phenomenon reported here are not well elucidated. One could hypothesize that serum levels of rituximab remain therapeutic for several months after completion of therapy. However, this rationale would not explain the late deepening of response seen in some patients for up to 7 years off therapy. A better suited hypothesis would be that the therapy administered is cytotoxic for most of the malignant clone. However, there could be more resistant, slow-cycling clones in which the effect of therapy is not immediately cytotoxic. These more resistant clones would receive a “kiss of death” by rituximab-containing therapy and then undergo cell death at a later time. Another hypothesis for this phenomenon would be a vaccine effect associated with rituximab therapy. It is well known that rituximab accumulates in patient’s serum after repeated infusions, such as in maintenance regimens. However, the effects reported here go beyond the time in which effective circulating rituximab concentrations are detected. In this scenario, rituximab may promote antigen uptake and peptide presentation by dendritic cells and leading to an antitumor response mediated by cytotoxic T-cells.

The oral Bruton tyrosine kinase inhibitor ibrutinib is approved in the United States and Europe for the treatment of patients with symptomatic WM, and is associated with high rates of response as well as durable PFS interval. It is unclear if the phenomenon of deepening of response after therapy completion presented here would also apply to WM patients treated with ibrutinib. Ibrutinib therapy is indefinite and should continue until disease progression or unacceptable toxicity. Therefore, WM patients do not complete therapy until disease progression. Furthermore, ibrutinib has a modulatory rather than cytotoxic effect on WM malignant cells, at least early in the course of therapy. This is supported by the deeper improvement observed in serum IgM levels, when compared to the degree of improvement in bone marrow involvement.

High bone marrow burden, presence of CXCR4 mutations and high serum IgM levels at the time of primary therapy initiation seem to be associated with lower odds of response, deepening after rituximab-containing therapy completion. The CXCR4 mutations have been associated with higher serum IgM levels, and possibly higher bone marrow disease burden in WM patients. Preclinical studies have suggested resistance to cell death induced by alkylating agents and proteasome inhibitors in CXCR4 mutated WM cell lines. However, a clinical study has suggested that CXCR4 mutations might not adversely impact the outcomes of WM patients treated with bortezomb. Additional research is needed to better understand the role, if any, that CXCR4 mutations play in drug resistance in WM patients.

Herein, we present a novel phenomenon of deepening of response after completion of therapy, which can be associated with better PFS and SAFTI in WM patients. Our results would need to be independently validated.

ACKNOWLEDGMENTS
Portions of this research have been presented at the 60th American Society of Hematology Meeting in San Diego, CA, in December 2018. Dr. Castillo would like to acknowledge the support of the WMR Fund.

CONFLICT OF INTEREST
JJC has received honoraria and/or research funds from Abbvie, Beigene, Janssen, Millennium, Pharmacyclics and TG Therapeutics. SPT has received research funding and/or consulting fees from Pharmacyclics and Janssen. All other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS
JJC designed the study and performed the analysis. JJC, JNG, AKe and KM collected patients’ data. GC, JC, MD, MLG, Ako, CJ, XL, MM, NT, CJP, LX, GY and ZRH performed molecular testing in patients’ samples. JJC, CAF, TED and SPT took care of patients. JJC and JNG drafted the manuscript. All authors critically reviewed and approved the final manuscript.

ORCID
Jorge J. Castillo https://orcid.org/0000-0001-9490-7532
REFERENCES


