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β_2-microglobulin Normalization Within 6 months of Ibrutinib-based Treatment is Associated with Superior PFS in CLL

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Abstract

High pre-treatment β_2-microglobulin (B2M) level is associated with inferior survival outcomes. However, the prognostic and predictive significance of changes in B2M during treatment have not been reported. We analyzed 83 patients treated with ibrutinib-based regimens (66 relapsed/refractory) and 198 treatment-naïve (TN) patients treated with combined fludarabine, cyclophosphamide and rituximab (FCR) to characterize change in B2M and their relationship to clinical outcomes. B2M rapidly fell during treatment with ibrutinib; in multivariable analysis (MVA), patients who received FCR [OR 0.40 (0.18–0.90), p=0.027] were less likely to normalize B2M at 6 months than patients treated with ibrutinib. On univariable analysis, normalization of B2M was associated with superior progression-free survival (PFS) from the 6-month landmark in patients treated with ibrutinib-based regimens and FCR. On MVA, failure to normalize B2M at 6 months of treatment was associated with inferior PFS [HR 16.9 (1.3–220.0), p=0.031] for ibrutinib-treated patients, after adjusting for the effects of baseline B2M, stage, fludarabine-refractory disease and del(17p). In contrast, in FCR-treated patients, bone marrow MRD-negative status was the only variable significantly associated with superior PFS [HR 0.28 (0.12–0.67), p=0.004]. Normalization of B2M at 6 months in ibrutinib-treated patients thus was a useful predictor of subsequent PFS and may assist clinical decision-making.

Keywords

CLL; chemoimmunotherapy; BTK-inhibitor; ibrutinib; beta-2 microglobulin

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Authorship.

PT designed research, collected and analyzed data and wrote the paper; LX and XW performed statistical analysis and co-wrote the paper; SOB, JAB, NJ, AF, ZE and MJK designed research and co-wrote the paper, WGW designed research, analysed data and wrote the paper.

Background

β_2-microglobulin (B2M) is the invariant peptide non-covalently associated with the HLA class I molecule, expressed on the surface of all nucleated cells.[1] Free B2M is found in body fluids under physiological conditions due to shedding from cell surfaces or intracellular release.[1, 2] It correlates with disease stage and tumor burden in patients with CLL.[3] Additionally, in patients who initially do not have indications for treatment, elevated B2M at diagnosis is independently associated with shorter time to first therapy.[4] B2M level correlates with CLL protein synthesis[5] and with markers of rapid proliferation such as elevated LDH, and CD38, and CD49d expression.[4] Thus, high B2M level in patients with early stage disease may reflect the presence of a more “activated”, proliferative clone.[6] When treatment is required, high B2M is independently associated with shorter survival.[7–11] Additionally, high levels may be associated with immune dysfunction through impaired ability to generate monocyte-derived dendritic cells.[1] B2M is renally excreted and may have improved predictive ability for treatment-free interval in patients with early stage CLL, when adjusted for GFR.[2] While initial B2M level is a well-established prognostic variable in CLL, correlations of patient outcomes with change in B2M during therapy have not been explored. We analyzed patients treated on investigational protocols at the MD Anderson Cancer Center (MDACC) from 2008–2013 to determine whether changes in B2M differed according to treatment regimen and whether normalization of B2M during treatment was predictive for or correlated with outcomes.

Methods

Patients treated on investigational protocols at MDACC between 2008 and 2013, with ibrutinib-based regimens (either as monotherapy or in combination with rituximab [IR] or BR [IBR]) or FCR, were included in these analyses, provided they had ≥3 months of follow-up and had at least a baseline and ≥1 subsequent B2M measurement. Patients treated with FCR or ibrutinib-based regimens were analyzed separately given the differences in the two patient populations. The upper limit of normal for B2M for the MDACC clinical laboratory was 2.3 mg/l. Follow-up B2M measurements were not mandated in these studies and were performed according to individual physician preference. All patients gave informed consent and studies were conducted in accordance with the declaration of Helsinki. The following variables were analyzed: baseline B2M, B2M at 6 months ±3 months, lowest level of B2M achieved and time to B2M normalization or nadir from start of treatment. Patients treated with FCR had end-of-treatment minimal-residual disease analysis performed on bone marrow using standardized 4-color flow cytometry, with a sensitivity of 0.01%, as previously described.[12] Statistical analyses were performed with SAS software V9.3 (SAS Institute Inc., Cary, NC), Splus Software V8.2 (TIBCO, Palo Alto, CA) and Graphpad Prism 6 (La Jolla, California). Descriptive statistics were used to summarize patient characteristics. Student t test was used to compare continuous variables that were normally distributed and the Mann-Whitney U test or Kruskal-Wallis test were used to compare continuous variables that were non-normally distributed. Dichotomous variables were compared using X^2 or Fisher’s exact tests. Multivariable analysis (MVA) for dichotomous outcome variables was performed using logistic regression. The probabilities of progression-free survival (PFS) and overall survival (OS) were estimated using the Kaplan-Meier method[13] and differences

between groups for each variable were assessed using the log-rank test.[14] MVA for survival were performed using the Cox proportional hazards model.[15] Variables with a p-value of <0.15 in univariable analyses (UVA) were evaluated in MVA. Progression-free survival was defined as the time from treatment initiation until progression according to IWCLL criteria,[16] next therapy or death. Six ibrutinib-treated patients and 4 FCR-treated patients who underwent planned allogeneic stem cell transplant while responding to therapy were censored for PFS analyses from the first day of the transplant conditioning but were followed for OS. Landmark PFS and survival analyses were performed from the time of 6-month B2M (+/− 3 months) to determine the prognostic significance of normalization of B2M at that time point. Not all patients had B2M measurements performed between 3 and 9 months after treatment initiation. These patients were included in descriptive analyses of change in B2M over time, but not in the landmark survival analyses. Given the association between renal function and B2M, we analyzed the association between estimated glomerular filtration rate (eGFR) and likelihood of B2M normalization and the association between eGFR, PFS and survival. eGFR was routinely calculated by our clinical laboratory according to the MDRD equation, using serum creatinine, age, sex and racial background of the patient.[17] A cut-off of <60mL/min/1.73m^2, corresponding to chronic kidney disease stage 3 was used to define “abnormal” renal function.[18]

Results

Patient characteristics

There were sufficient B2M results and duration of follow-up for 83 ibrutinib-treated (35 ibrutinib monotherapy, 37 IR, 11 IBR) and 198 FCR-treated patients for this analysis. Baseline characteristics according to treatment group are shown in Table 1. Median duration of follow-up was 23.8 months (range 5.5–45.1) for ibrutinib-treated patients and 41.5 months (range 14.4–69.1) for FCR-treated patients. Consistent with the fact that patients treated with ibrutinib-based regimens were relapsed/refractory, median baseline B2M was higher in ibrutinib- compared to FCR-treated patients (4.1 vs. 3.6, respectively, p=0.02) and more patients treated with ibrutinib had del(17p) (42.2% vs. 5.6%, p <0.001), unmutated immunoglobulin heavy chain variable *IGHV* gene (69.9% vs. 53.5%, p<0.001) and advanced Rai stage (56.7% vs. 41.0%, p = 0.02). Ibrutinib-treated patients were older than those treated with FCR (median age 67 and 59, respectively), p <0.001.

Association between baseline B2M and other baseline variables

There was a significant association between baseline B2M of ≥4.0mg/l and the following variables in the UVA: Rai stage III–IV, bulky lymphadenopathy (lymph node ≥5 cm), del(17p) and eGFR <60ml/min/1.73m^2 [OR 3.75 (2.07–6.78), p<0.001]. There was no association between del(11q), *IGHV* mutation status, gender or ZAP70 expression and baseline B2M ≥4.0mg/l. In MVA, all four of the following characteristics remained independently associated with baseline B2M ≥4.0mg/l: del(17p) [OR 2.27 (1.18–4.40), p=0.02], eGFR <60ml/min/1.73/m^2 [OR 4.87 (2.56–9.29), p<0.001], bulky adenopathy [OR 3.45 (2.07–5.78), p<0.001], and Rai stage III–IV [OR 3.13 (1.87–5.24), p<0.001].

Change in B2M and absolute lymphocyte count with time

There was no correlation between pre-treatment B2M and absolute lymphocyte count (ALC). Despite the expected treatment-associated lymphocytosis, which predictably occurred upon ibrutinib initiation, there was a concurrent rapid reduction in B2M. In contrast, FCR treatment was associated with a rapid reduction in ALC, which preceded changes in B2M (Figure 1A). We compared changes in ALC and B2M over time in patients treated with ibrutinib monotherapy or IR. Similar B2M reduction is seen with each regimen, despite a much more rapid reduction in ALC with IR. There were too few measurements of B2M in patients treated with IBR to analyze those patients separately (Figure 1B).

Association between normalization of B2M after 6 months of treatment and baseline patient characteristics

The baseline characteristics associated in UVA with likelihood of normalizing B2M (<2.4 mg/l) at 6 months in ibrutinib-treated patients are shown in Table 3. In MVA, age ≥65 [OR 0.07 (0.01–0.89), p=0.04], baseline B2M ≥4.0mg/l [OR 0.01 (0.0–0.11), p=0.001] and fludarabine-refractory disease [OR 0.01 (0.0–0.28), p=0.008], were all strongly associated with lower likelihood of normalizing B2M at 6 months of treatment.

The baseline characteristics associated in UVA with likelihood of normalizing B2M (<2.4mg/l) at 6 months in FCR-treated patients, are shown in Table 4. In MVA, age ≥65 [OR 0.26 (0.08–0.80), p=0.02], baseline B2M ≥4.0mg/l [OR 0.23 (0.10–0.52), p<0.001] and eGFR <60ml/min/1.73m^2 [OR 0.36 (0.13–0.99), p=0.048], were all significantly associated with lower likelihood of normalizing B2M at 6mo.

In MVA, including those baseline characteristics significantly associated with likelihood of B2M normalization at 6 months, patients who received FCR [OR 0.40 (0.18–0.90), p=0.027] were less likely to normalize B2M at 6 months than patients treated with ibrutinib (Table 2).

Association between normalization of B2M at 6 months and depth of best response

The overall response rate (ORR) for ibrutinib-treated patients was 92.8%; 13.3% achieved complete remission (CR). This higher-than-expected CR rate was due to a higher number of CRs in those patients receiving IBR. There was no association between normalization of B2M at 6 months and final response rate; 50% of patients who achieved CR normalized their B2M at 6mo, compared to 49.1% of patients who achieved partial remission (PR) (p=0.924). The ORR for FCR-treated patients was 96.5%, 67.7% achieved CR. In contrast to ibrutinib-treated patients, there was a significant association between depth of response and B2M normalization in FCR-treated patients; 54.4% of patients who achieved CR normalized B2M at 6 months, versus 35.6% of those who achieved PR (p=0.035).

Relationship between MRD-negative status and B2M in FCR-treated patients

Despite the association between CR and B2M in FCR-treated patients, there was no association between achieving bone marrow MRD-negative status and normalization of B2M; 53.6% of 84 FCR-treated patients who were MRD-negative had a B2M <2.4mg/l at 6mo, while 47.9% of patients who were MRD-positive had a B2M <2.4mg/l. There were 37 patients treated with FCR who had simultaneous measurement of B2M (20 after cycle 3 and

17 after cycle 6 of FCR) and bone marrow MRD by flow cytometry. Twenty-four patients who achieved MRD-negativity in bone marrow still had elevated B2M at the time of MRD-assessment; median B2M was 2.8mg/l (range 1.5–6.9) at the time of achieving MRD-negative status. Fourteen of 24 MRD-negative patients with initially abnormal B2M subsequently normalized their B2M in the absence of salvage therapy at a median time of 14 months after initiation of treatment (range 5–41 months).

Associating change in B2M with progression-free survival

Landmark analysis was performed to evaluate the association between B2M normalization at 6 months (+/− 3 months) and PFS. In ibrutinib-treated patients, normalization of B2M at 6 months was associated with superior PFS from the landmark point (median PFS 22.3 months vs. NR, p=0.042) (Figure 2). The only other baseline factor associated with inferior PFS in UVA in ibrutinib-treated patients was fludarabine-refractory disease (median PFS 15 months vs. NR, p=0.009) (Figure 2); there was a trend towards inferior PFS in patients with del(17p), (median PFS 22.2 months vs. NR, p=0.059) and baseline B2M ≥4.0mg/l (p=0.054). MVA was performed, including B2M normalization and fixed pre-treatment characteristics demonstrated to be significantly associated with PFS in 6 month landmark analysis. In MVA, only achieving B2M normalization at 6 months was significantly associated with PFS [HR 16.9 (1.3–220.0), p=0.031]; there was a trend toward inferior PFS in patients with baseline B2M ≥4.0mg/l [HR 0.12 (0.01–1.13), p=0.064] and del(17p) [HR 3.09 (0.71–13.28), p=0.13].

In FCR-treated patients, normalization of B2M at 6 months (+/− 3 months) was also associated with superior PFS (median PFS 53.8 months vs. NR, p=0.004) (Figure 3). Pre-treatment characteristics associated with superior PFS from the 6-month landmark were baseline B2M ≥4.0mg/l (median PFS 42.8 months vs. NR, p<0.001), del(17p), (median PFS 38.5 months vs. NR, p<0.001), unmutated *IGHV* (median 42.8 months vs. NR, p<0.001), ZAP70 expression (median PFS 44.6 months vs. NR, p<0.001) and bone marrow MRD-negative status (median PFS 44.1 months vs. NR, p<0.001). However, in MVA, only bone marrow MRD-negative status was significantly associated with longer PFS [HR 0.28 (0.12–0.67), p=0.004] (Figure 3).

There was no significant difference in PFS in patients who were MRD-negative versus positive by B2M at 6 months <2.4 or ≥2.4mg/l, but the number of events was small. In contrast, in patients who were MRD-positive, patients with B2M <2.4mg/l had a longer PFS compared to those with B2M ≥2.4mg/l at 6 months [median PFS not reached (NR) vs. 34.4mo, p=0.037], data not shown.

Associating changes in B2M with OS

There was no significant difference in OS according to normalization of B2M in patients treated with ibrutinib-based regimens or FCR. However, in ibrutinib-treated patients, fludarabine-refractory disease (median survival 18.2 months vs. NR, p=0.001) and del(17p), (median survival 22.2 months vs. NR, p=0.02) were significantly associated with shorter survival. In MVA, only fludarabine-refractory disease was significantly associated with survival [HR 4.1 (1.4–11.9), p=0.009]. In FCR-treated patients, baseline B2M ≥4.0mg/l

(p=0.009), del(17p), (median OS 41.1 vs. NR, p=0.0006), unmutated *IGHV* (p=0.006) and failure to achieve MRD-negative status (p=0.0097) were all significantly associated with inferior survival in UVA. In MVA, only MRD-negative status was significantly associated with longer survival [HR 0.28 (0.12–0.67), p=0.004].

Timing of rise in B2M and relapse

Patients who subsequently relapsed were analyzed to determine if rise in B2M (considered significant if increased by ≥20% of the nadir level) was predictive of subsequent relapse. B2M rise did not reliably precede clinical relapse; more frequently, it occurred simultaneously with clinical relapse; as such, serial B2M does not appear to be a useful predictor of clinical relapse (data not shown).

Conclusions

Pre-treatment characteristics significantly associated with risk for shorter PFS in FCR-treated patients were previously described and include high pre-treatment B2M, unmutated *IGHV*, del(17p) and high baseline ALC[10]. Furthermore, del(17p), high B2M and poor performance status were associated with shorter OS.[10] Del(17p) was associated with shorter PFS in ibrutinib-treated patients, despite a similar ORR in patients with non-del(17p) CLL.[19] The significance of elevated pretreatment B2M is well-established. Here, for the first time, we demonstrated the prognostic importance of B2M normalization during therapy, particularly in ibrutinib-treated patients.

Ibrutinib is associated with transient lymphocytosis on treatment initiation;[19, 20] however, B2M fell rapidly within the first few months of treatment, despite the associated lymphocytosis; normalization of B2M was achieved in ibrutinib-treated patients despite measurable persistent disease in the majority of patients. In fact, patients treated with Ibrutinib were more likely to achieve normalization of B2M levels within 6 months of treatment than those treated with FCR, the majority of whom had achieved CR by the 6-month time point. Patients treated with FCR frequently had persistently elevated B2M, despite achieving bone marrow MRD-negative CR. In addition, normalization of B2M may be delayed until many months after completion of therapy in FCR-treated patients. The reasons for these differences are not clear.

Most patients who normalized B2M during ibrutinib treatment still had significant residual disease (i.e. had not achieved CR). This raises the possibility that the reduction in B2M during ibrutinib therapy may not be solely due to a reduction in tumor burden. Instead, a potential hypothesis is that ibrutinib may reduce production and/or shedding of B2M into plasma from CLL cell surfaces, through an as-yet uncharacterized mechanism.[21]

MRD-negative status after treatment with FCR is a powerful predictor of longer survival outcomes.[22, 23] However, MRD assessment is not useful in ibrutinib-treated patients, given the low CR rate[19] and very low rate of MRD-negative remission.[24] We showed that normalization of B2M at 6 months of ibrutinib-based treatment was associated with superior PFS, independent of pre-treatment prognostic features. This simple tool may thus help early identification of patients at greater risk for subsequent disease progression.

In FCR-treated patients, where MRD assessment after treatment is a well-established method of identifying patients at higher risk of progression, serial B2M measurement may provide additional information regarding risk of progression in patients with persistent disease in the marrow. Those patients whose B2M normalized had a superior outcome to those with persistently elevated B2M. Determination of whether B2M normalization has similar prognostic relevance in MRD-negative patients after FCR treatment will require analysis of larger patient groups.

Our study has some limitations: firstly, B2M measurements were not prescribed in these protocols and as such were not performed in all patients or at identical time points. Secondly, the patient population was heterogeneous; FCR-treated patients were treatment-naïve and had more favorable baseline characteristics and longer follow-up; additionally, the ibrutinib-treated group consisted of both treatment-naïve and relapsed/refractory patients and contained patients treated with both single-agent and combination therapy. The heterogeneity of the patients within this study, however, increases the generalizability of our findings; normalization of B2M at 6 months after commencing treatment appears to be a robust predictor of PFS, being independently associated with PFS in ibrutinib-treated patients, regardless of baseline B2M, other baseline prognostic markers and depth of response post-treatment. Finally, numbers of patients were insufficient to stratify analysis for important prognostic variables such as del(17p); treatment decisions in this group of patients are complex, particularly the decision of whether to proceed with allogeneic stem cell transplantation in patients responding to ibrutinib; the availability of a dynamic marker of prognosis akin to MRD assessment may assist clinical decision-making.

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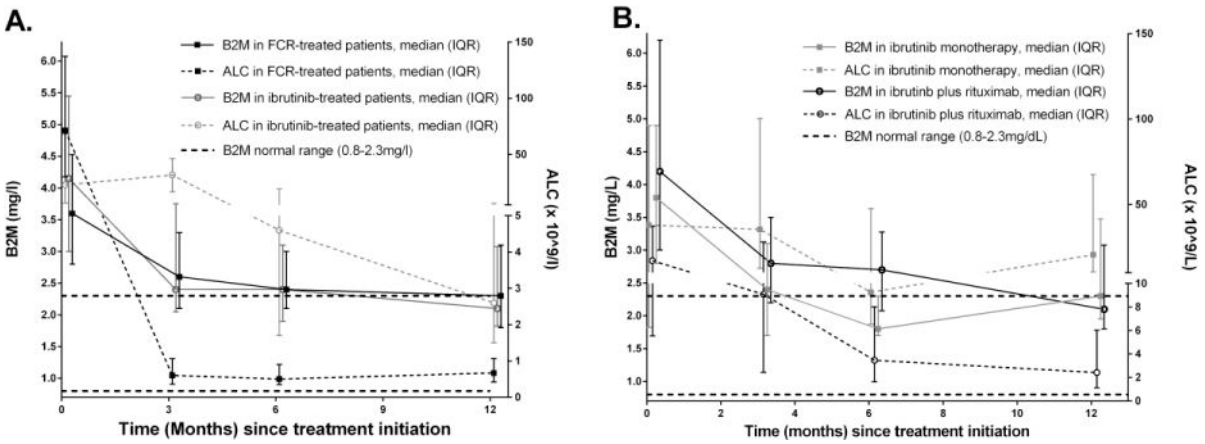

Figure 1.

A - Median β_2-microglobulin (B2M) and absolute lymphocyte count (ALC) over time in patients treated with ibrutinib-based regimens or fludarabine, cyclophosphamide and rituximab (FCR). Ibrutinib-based regimens produced rapid reduction in B2M despite persistent lymphocytosis. Median and IQR (interquartile range) shown. Number of measurements at each timepoint are: Ibrutinib-based regimens; baseline 83, 3 months 54, 6 months 53, 12 months 52); FCR (baseline 198, 3 months 103, 6 months 100, 12 months 68). B - Median β_2-microglobulin (B2M) and absolute lymphocyte count (ALC) over time in patients treated with ibrutinib monotherapy or ibrutinib plus rituximab. Similar B2M reduction is seen with each regimen, despite a much more rapid reduction in ALC with ibrutinib plus rituximab. Number of measurements at each timepoint are: Ibrutinib; baseline 35, 3 months 13, 6 months 15, 12 months 8; ibrutinib plus rituximab; baseline 37, 3 months 36, 6 months, 35, 12 months, 35.

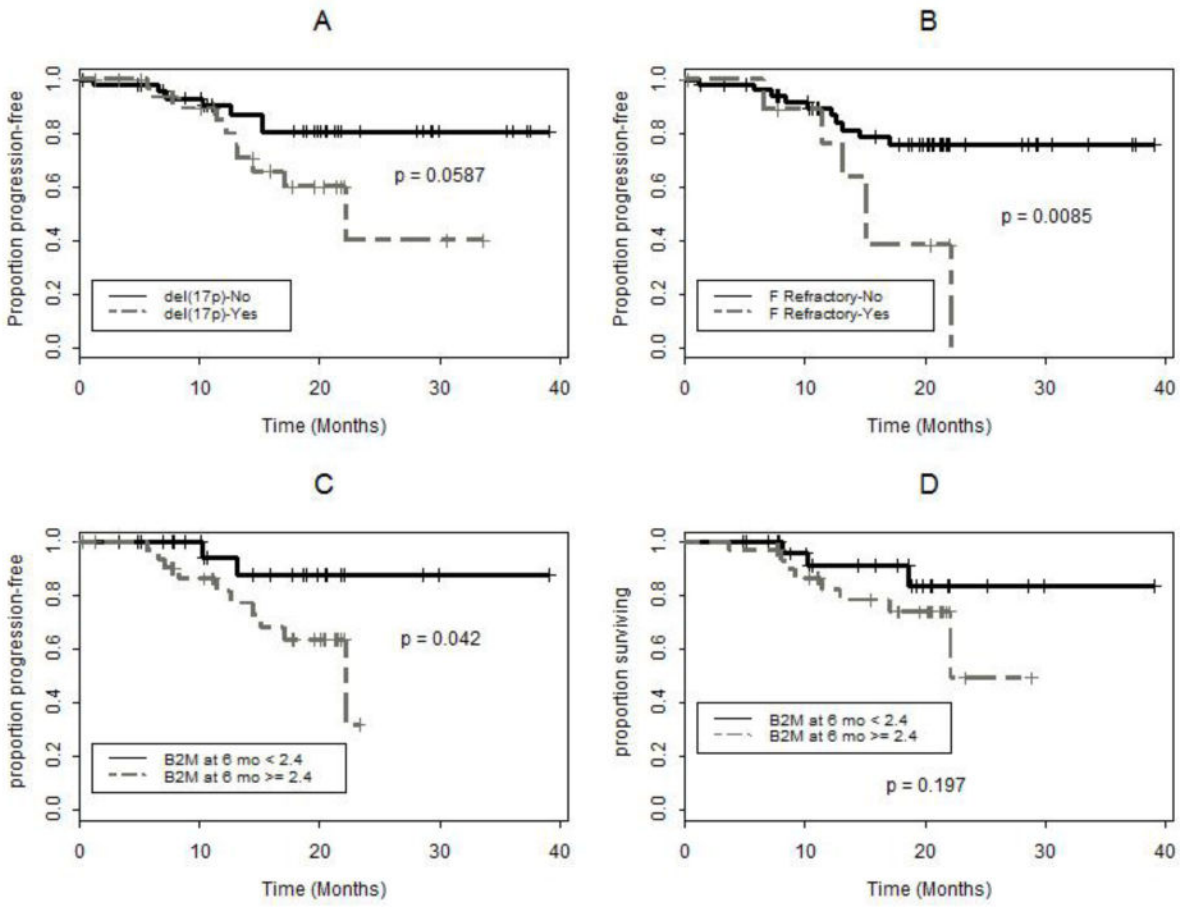

Figure 2.

Survival outcomes in ibrutinib-treated patients: A) Progression-free survival (PFS) according to presence or absence of del(17p); B) PFS according to presence of absence of fludarabine-refractory disease; C) PFS according to normalization of B2M at 6 months versus not; D) Overall survival according to normalization of B2M at 6 months versus not. Abbreviations: B2M, β_2-microglbulin; F, fludarabine.

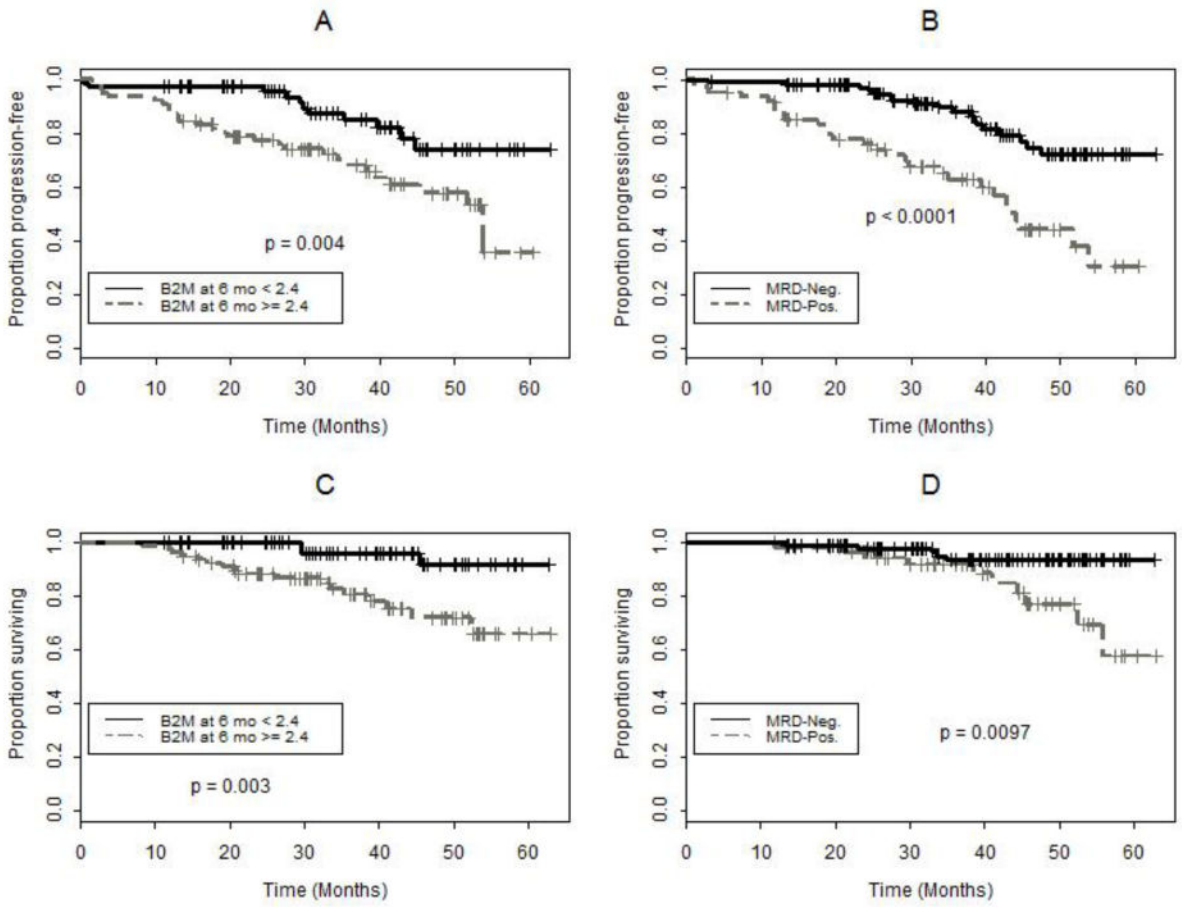

Figure 3.

Survival outcomes in fludarabine, cyclophosphamide and rituximab (FCR)-treated patients: A) Progression-free survival (PFS) according to normalization of B2M at 6 months or not; B) PFS according to MRD-negative status at completion of therapy; C) OS according to normalization of B2M at 6 months or not; D) OS according to MRD status at completion of therapy. Abbreviations: B2M, β_2-microglbulin; MRD, minimal residual disease.

Table 1

Patient baseline characteristics by treatment regimen.

Baseline Characteristic	Ib (n=83)	FCR (n=198)	P-value
Age, median (range)	65 (35–82)	59 (32–79)	0.001
Male, n (%)	56 (68)	121 (61)	0.332
Previously-treated n (%)	66 (80)	0	<0.001
Rai stage, n (%)			
I–II	36 (43)	118 (60)	
III–IV	47 (57)	80 (40)	0.011
Bulky adenopathy (>/=5cm), n (%)	38 (46)	96 (49)	0.817
FISH hierarchy, n (%)			
Del(13q)	13 (16)	65 (33)	
Negative	8 (10)	47 (24)	
Trisomy 12	6 (7)	34 (17)	
Del(11q)	21 (25)	39 (20)	
Del(17p)	35 (42)	11 (6)	<0.001
***IGHV* mutation status, n (%)**			
Mutated	9 (11)	71 (36)	
Unmutated	58 (70)	106 (54)	
Unknown	10 (12)	21 (11)	<0.001
ZAP70 positive, n (%)	48 (71)	119 (65)	0.716
B2M, quartile			
1st	1.8–3.0	1.2–2.8	
2nd	3.0–4.1	2.8–3.6	
3rd	4.1–5.3	3.6–4.5	
4th	5.3–14.0	4.5–12.5	0.02
B2M ≥4.0mg/l, n (%)	47 (56.6)	82 (41.4)	0.047
Number of B2M measurements between baseline and 1 year, n (%)			
0	1 (1)	7 (4)	
1	11 (13)	79 (40)	
2	14 (17)	74 (37)	
≥3	57 (69)	38 (19)	<0.001
eGFR (ml/min/1.73m^2):			
30–59	18 (22)	39 (20)	
60–89	51 (61)	130 (66)	
≥90	14 (17)	29 (15)	0.808

Ib, Ibrutinib-based regimens: Ibrutinib monotherapy, ibrutinib plus rituximab or ibrutinib, bendamustine and rituximab; FCR, Fludarabine, cyclophosphamide and rituximab; eGFR, estimated glomerular filtration rate; IGHV, immunoglobulin heavy chain variable gene; B2M, beta-2 microglobulin.

Table 2

B2M at different time points according to treatment group.

Treatment group	n	Median (IQR) B2M (mg/l) @ Baseline	Median (IQR) B2M (mg/l) @ 6 months	Median (IQR) B2M (mg/l) @ Nadir
All Ibrutinib (n = 83)	83	4.1 (3.0–5.0)	2.4 (1.9–3.1)	2.1 (1.8–2.9)
Ibrutinib monotherapy	35	4.0 (3.0–5.0)	2.0 (1.6–2.5)	2.0 (1.7–2.8)
IR or IBR	48	4.2 (3.0–5.9)	2.6 (2.0–3.4)	2.1 (1.8–2.9)
Treatment-naïve	17	3.8 (2.9–4.5)	2.1 (1.8–2.5)	1.9 (1.8–2.2)
Relapsed/refractory	66	4.4 (3.0–6.0)	2.5 (1.0–3.4)	2.1 (1.8–3.1)
FCR	198	3.6 (2.8–4.5)	2.4 (2.0–3.0)	2.0 (1.6–2.6)

IQR, interquartile range; FCR, fludarabine, cyclophosphamide and rituximab; B2M, β_2-microglobulin; n, number; IR, ibrutinib plus rituximab; IBR, ibrutinib, rituximab, bendamustine

Table 3

Likelihood of normalizing B2M at 6 months according to baseline characteristics in Ibrutinib-treated patients.

Variable	Value	n	B2M normal at 6mo, n(%)	P-value
Age (yrs)	<65	32	19 (59)	0.08
	≥65	39	15 (39)	
Sex	Female	25	10 (40)	
	Male	46	24 (52)	0.33
Baseline B2M (mg/l)	<4.0	30	24 (80)	
	≥4.0	67	10 (24)	<0.001
Baseline eGFR (ml/min/1.73m^2)	≥60	58	30 (52)	
	<60	13	4 (31)	0.17
Rai stage	0–II	29	19 (66)	
	III–IV	42	15 (36)	0.01
Maximum lymph node size at baseline (cm)	<5	34	20 (59)	
	≥5	37	14 (38)	0.08
Fludarabine-refractory	Not refractory	46	22 (48)	
	Refractory	10	1 (10)	0.03
Del(17p) by FISH	No	40	22 (55)	
	Yes	31	12 (39)	0.17
***IGHV* mutation status**	Mutated	9	6 (67)	
	Unmutated	49	21 (43)	0.14
ZAP70 expression	Negative	18	8 (44)	
	Positive	44	22 (50)	0.69

eGFR, estimated glomerular filtration rate; B2M, β_2-microglobulin; *IGHV*, Immunoglobulin heavy chain variable gene; n, number.

Table 4

Likelihood of normalizing B2M at 6 months in FCR-treated patients.

Variable	Value	n	B2M normal at 6mo, n(%)	P-value
Age (yrs)	<65	137	68 (50)	0.006
	≥65	28	6 (21)	
Sex	Female	68	37 (54)	
	Male	97	37 (38)	0.039
Baseline B2M (mg/l)	<4.0	98	60 (61)	
	≥4.0	67	14 (21)	<0.001
Baseline eGFR (ml/min/1.73m^2)	≥60	133	67 (50)	
	<60	32	7 (22)	0.004
Rai stage	0–II	96	47 (49)	
	III–IV	69	27 (39)	0.21
Maximum lymph node diameter at baseline (cm)	<5	84	44 (52)	
	≥5	79	30 (38)	0.07
Del(17p) by FISH	No	7	1 (14)	
	Yes	156	72 (46)	0.097
***IGHV* mutation status**	Mutated	60	31 (52)	
	Unmutated	88	35 (40)	0.34
ZAP70 expression	Negative	53	27 (41)	
	Positive	99	39 (59)	0.17

Abbreviations: eGFR, estimated glomerular filtration rate; B2M, β_2-microglobulin; *IGHV*, Immunoglobulin heavy chain variable gene; n, number.