Long-term outcomes by bone marrow B-cell depletion from the R2W trial of bortezomib with cyclophosphamide and rituximab in Waldenstrőm macroglobulinaemia

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Abstract

There remains a lack of consensus as to the most appropriate primary therapy in Waldenstrőm macroglobulinemia (WM). We evaluated a novel bortezomib-based combination and developed a sensitive WM-specific flow cytometry assay (limit of detection 0.004% of leucocytes) to assess bone marrow (BM) response. Sixty treatment-naïve WM patients were enrolled into this phase II trial and randomised (2:1) to receive cyclophosphamide and rituximab with either bortezomib (BRC) or fludarabine (FCR). The primary objective was to assess the overall response rate (ORR) in eligible patients receiving BRC (N=41). An ORR of 97.6% (95%CI:87.1-99.9) was observed; 27 (65.9%) patients remain alive without progression after 62.6 months median follow-up, with 2-, 3- and 5-year progression-free survival (PFS) rates of 92.7% (95%CI:79.0-97.6), 80.5% (95%CI:64.8-89.7) and 65.5% (95%CI:48.8-77.9). Persistent WM B-cells were demonstrable in 19/38 patients at the end of treatment (median 0.24%, range 0.02-11.2%). PFS was markedly longer in patients with BM B-cell depletion (<0.004%) compared to those who had persistent BM B-cells detectable at end of treatment (HR=0.06, 95%CI:0.01-0.47, p<0.001), and remained independently associated after adjusting for baseline risk stratification or investigator-assessed response. BRC is a tolerable, highly efficacious regimen for treatment-naïve WM patients. BM B-cell depletion is independently associated with patient outcomes.

Introduction

Pathologically, Waldenstrőm macroglobulinaemia (WM) comprises two main cellular components: a B-cell component which is likely to be proliferative and CD20 expressing and a plasma cell component responsible for the IgM secretion but non-proliferative and CD20 negative. Rituximab-based immunochemotherapy regimens remain the standard of care in the first-line treatment of patients [1-3] despite the emergence of BTK inhibitors (BTKi). Dexamethasone, rituximab and cyclophosphamide (DRC) and bendamustine plus rituximab (BR) are widely used, highly efficacious, first-line regimens but these target and selectively deplete the B-cell component rather than plasma cells. As such they can be associated with slow and delayed responses in IgM. [4,5] Bortezomib-based combinations are an additional option and may provide more rapid IgM responses possibly as a consequence of targeting the plasma cell component of the disease.[6, 7] In R2W, we assessed a novel bortezomib combination of weekly subcutaneous bortezomib with rituximab and cyclophosphamide (BRC) specifically developed to target the different cellular components of the disease.

While the depth of clinical response following rituximab-based therapy has been shown to predict progression-free survival (PFS) [8] the value of the traditional response assessments is impacted by a number of factors. These include kinetics of response, which may vary by regimen, as well as the fact that major response rates are high with most modern regimens but complete responses remain rare. As a consequence, conventional response categories may not provide the optimal prediction of survival outcomes which can be long, at least in the front-line setting. Cellular or molecular

based response evaluation may therefore provide a better outcome measure. Varettoni and colleagues have recently reported that molecular remission (based on *MYD88*) predicted for PFS in a retrospective analysis of the *FIL_BIOWM* study. [9] In this study we have developed a novel, highly sensitive and disease-specific flow cytometric assay that allows quantitative assessment of B-cell response in both blood and bone marrow (BM), compared this with conventional IgM responses and assessed its impact on PFS. It should be noted that numerous studies of this kind in chronic lymphocytic leukaemia (CLL) [10] and myeloma [11] have shown that the quantitation of marrow response in this way is more predictive of survival outcome (overall survival [OS] as well as PFS) than conventional response criteria in multivariable models.

Subjects and Methods

Patients

Treatment-naïve patients with symptomatic WM were enrolled into R2W, a prospective randomised (2:1), multicentre, non-comparative phase II trial (Clinicaltrials.gov identifier: NCT01592981). Patients aged ≥18 years with a confirmed diagnosis of WM according to World Health Organisation (WHO) criteria and ECOG Performance Status 0-2 were included. BM biopsy was performed in all patients and the pathological diagnosis established following central review. Complete eligibility and exclusion criteria are available in the protocol. The study was approved by the NRES Committee London – City and East and managed by the Cancer Research UK and University College London Cancer Trials Centre. Written informed consent was obtained in accordance with the Declaration of Helsinki. Patients were stratified according to the International Prognostic Scoring System for WM (IPSSWM).

Study design and treatment

The R2W trial comprised a safety run-in with BRC for the first six patients (Supplementary Materials), followed by randomisation to BRC or fludarabine, rituximab and cyclophosphamide (FCR) (2:1); FCR was considered a standard of care at the time of trial conception and design, and was therefore included as a contemporary control arm for reference purposes. The BRC regimen consisted of bortezomib 1.6 mg/m^2 subcutaneously (SC) on days 1, 8, 15; cyclophosphamide 250 mg/m^2 orally on days 1, 8, 15 and rituximab 375 mg/m^2 intravenously (IV) on days 1, 8, 15, 22 of cycles 2 and 5 only. The FCR regimen consisted of fludarabine 40mg/m^2 orally on days 1-3; cyclophosphamide 250 mg/m^2 orally on days 1-3 and rituximab 375 mg/m^2 IV on days 1, 8, 15, 22 of cycles 2 and 5 only. If the oral preparation of cyclophosphamide was not tolerated, intravenous administration of cyclophosphamide was permitted at the daily dose of 200 mg/m^2. Rituximab was delayed until cycle 2 in both treatment arms to mitigate the risk of IgM associated rebound hyperviscosity. Regimens were repeated every 28 days for up to six cycles, following which patients were observed for up to five years.

Dose modifications and delays of therapy were permitted as per protocol. Transfusion and growth factor support was permitted at the investigator's discretion in accordance with local procedures. In the event of a patient developing bortezomib-related neuropathic pain or peripheral sensory neuropathy, treatment was delayed for up to two weeks. If the toxicity did not resolve during this hold period, bortezomib was discontinued; if it resolved to grade ≤1, bortezomib was restarted at a reduced dose of 1.3 mg/m^2. If symptoms recurred, restart of bortezomib was permitted at 1.0 mg/m^2

after a second hold period and at 0.7 mg/m^2 after a third hold period; bortezomib was permanently discontinued if a fourth hold period was required or if a patient experienced grade 4 toxicity. Prophylaxis with acyclovir was recommended for patients in both arms and continued for at least six months after completing trial treatment. Patients in the FCR arm were recommended to receive co-trimoxazole concurrently.

Assessments

Response evaluation with paraprotein quantification and quantitative serum immunoglobulins was performed prior to each cycle, three to four weeks after day one of cycle six and then every three months for up to five years. Computed tomography (CT) of the neck, chest, abdomen and pelvis was performed at baseline and only repeated if abnormal. Response determinations were made using consensus panel criteria from the VIth International Workshop on WM [12] and were determined on an intention-to-treat basis. Quality of life (QoL) questionnaires (EQ-5D) were completed at baseline and after three and six cycles of treatment.

BM aspirate and peripheral blood samples were obtained at baseline, following three cycles and at three months after the last cycle of treatment and assessed for WM-specific B-cells, phenotype CD22(+wk)/CD25+/CD200(+wk)/IgM+/CD305-/CD185(+wk), in a single laboratory using multiparameter flow cytometry (limit of detection 0.004%). Cell pellets containing 10^6 leukocytes were incubated with pre-prepared antibody cocktails including CD19, CD20, CD45, CD5, CD10, CD200, CD305, CD22, CD25, CD185, Kappa and Lambda. A minimum of 500,000 cells were acquired and analysed for each antibody combination using a Canto II flow

cytometer with FACSDiva software (BD Biosciences). We followed conventional and agreed definitions to define the sensitivity of our assay; 20 events were needed to define the limit of detection (0.004%) whilst 50 events were considered necessary for the limit of quantitation (0.01%). Patient cases were classified as having residual neoplastic B-cells if a discrete population of phenotypically aberrant B-cells comprising ≥20 events was identified in the 500,000-cell file.

Presence of the characteristic *L265P* mutation of *MYD88* was assessed on all baseline samples using allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) which has a sensitivity of 5%. Additional targeted sequencing of *MYD88* was performed using a high-throughput methodology on a MiSeq system (Illumina, Chesterford, UK).

Statistical considerations

The primary endpoint of this phase II trial was end of treatment investigator-assessed overall response rate (ORR). In eligible patients treated with BRC, an ORR of 80% was targeted whilst a rate <60% would be of no further interest. Following the 6-patient safety run-in, a further 33 patients were required to assess ORR using a three-outcome single-stage design [13] with 90% power and one-sided 5% significance level. An additional 17 patients were randomised to an internal control arm (i.e. allocation ratio 2:1), stratified by IPSSWM.

Secondary endpoints include safety, compliance, QoL, PFS, time to next treatment (TTNT) and OS. Statistical analyses were primarily descriptive. Time-to-event endpoints were estimated using Kaplan-Meier methods, measured from registration

with patients censored at the date last seen; Cox regression was used to explore associations with IPSSWM, revised (r)IPSSWM, investigator-assessed response and BM B-cell depletion. All eligible patients who completed response assessments or stopped treatment early due to toxicity or insufficient response are included in the primary efficacy analysis.

Results

Patient characteristics

Following successful completion of the safety run-in, during which none of the initial six patients treated with BRC (250 mg/m^2 cyclophosphamide) experienced a dose-limiting toxicity, a further 54 patients were randomised. The CONSORT flow diagram is shown in Figure 1. In total, 60 patients were enrolled from 24 UK centres (Supplementary Table 1) between January 2013 and September 2015. Here, we focus on the investigational BRC group (N=43) whilst data for the FCR (N=17) internal control group are presented in the Supplementary Materials.

Baseline characteristics for the BRC group are shown in Table 1; 29 (67.4%) patients were male and the median age was 67 years (range 43-78). Ten (23.3%), 15 (34.9%) and 18 (41.9%) were IPSSWM low, intermediate, and high risk respectively; 5 (11.9%), 10 (23.8%), 12 (28.6%), 11 (26.2%) and 4 (9.5%) were rIPSSWM very low, low, intermediate, high, and very high risk respectively (*1 missing*). Median (range) haemoglobin was 9.8 g/dL (6.5-14.0) and serum IgM paraprotein 32.0 g/L (3.2-80.2). Clinical symptoms of hyperviscosity were present in 28 (65.1%), see Table 1 for details. Lymphadenopathy was present in 12 (27.9%), B symptoms in 9 (20.9%),

splenomegaly in 7 (16.3%) and peripheral neuropathy in 8 (18.6%) patients prior to starting treatment. The *MYD88 L265P* mutation was demonstrable in 36/38 (94.7%) evaluable patients.

One BRC patient withdrew before starting trial treatment, therefore 42 patients are included in the safety population, and one BRC patient was found to be ineligible and withdrawn during cycle one and thus excluded from the efficacy analyses (N=41).

Efficacy of BRC

End of treatment ORR in the BRC group (N=41) was 97.6% (95% CI: 87.1-99.9), exceeding the target rate of 80%; 1 (2.4%) patient achieved CR, 8 (19.5%) VGPR, 24 (58.5%) PR and 7 (17.1%) MR, whilst 1 (2.4%) patient had SD. The median relative change from baseline in serum IgM was -84.9% (range: -99.7% to -47.1%), and a median time to a 50% reduction in serum IgM of 4.3 months (95% CI: 3.2-5.7). Median time to best response was 26.9 months (95% CI: 18.0-38.7).

After a median follow-up of 62.6 months, 27 (65.9%) are alive without progression, 12 (29.3%) are alive having progressed and two (4.9%) have died. Two-, 3- and 5-year PFS rates in the BRC group are 92.7% (95% CI: 79.0-97.6), 80.5% (95% CI: 64.8-89.7) and 65.5% (95% CI: 48.8-77.9) respectively (Figure 2A). Eight patients have received further treatment for progression, four received rituximab and bendamustine, one received rituximab-only, and three received ibrutinib. Two-, 3- and 5-year TTNT rates in the BRC group are 97.6% (95% CI: 83.9-99.7), 95.0% (95% CI: 81.4-98.7) and 79.4% (95% CI: 63.0-89.1) respectively (Figure 2B).

Two (4.9%) BRC patients have died, due to pulmonary embolism and cardiac failure respectively, giving a 5-year OS rate of 95.0% (95% CI: 81.4-98.7), (Figure 2C). QoL is described in the Supplementary Materials.

Flow cytometric response evaluation of bone marrow B-cell status

The flow cytometric assay that we developed built upon the WM-specific B cell phenotype described by Paiva et al (CD22dim CD25+).[14] The assay has a limit of detection of 0.004% ($4x10^{-5}$) in adequate samples but sensitivity was more limited in haemodilute BM samples, which were reported as inadequate. The assay specifically detects and enumerates WM B-cells only. WM plasma cells cannot currently be assessed in this way as a WM-specific plasma cell phenotype has not been described and, as such, WM plasma cells cannot be reliably distinguished from normal plasma cells.

Neoplastic B-cells with a WM-phenotype were found in the BM of all evaluable patients at baseline. Low level peripheral blood involvement was noted in 43/54 (79.6%) patients with median neoplastic B-cells of 1.66% of leucocytes, absolute count 0.094 x 10^9/l (0.01-36%, 0.005-1.5 x 10^9/l). Persistent WM B-cells were demonstrable in BM aspirate samples of 21/33 (63.6%) BRC patients (median 1.05%, range 0.04-32.0%) following three cycles and in 19/38 (50.0%) patients (median 0.24%, range 0.02-11.2%) at the end of BRC therapy. Of the 19 patients with no detectable B cells in the bone marrow at the end of treatment, 1 achieved CR, 6x VGPR, 7x PR, 5x MR and 0x SD; median relative change from baseline in serum IgM was -88.8% (range: -98.1% to -53.9%). There was no difference in IPSSWM (p=0.52) or rIPSSWM (p=0.43) in patients with/without detectable B cells in the bone marrow at the end of treatment.

Residual circulating neoplastic B-cells could be demonstrated in the peripheral blood of a much lower proportion of patients: 4/37 (10.8%) patients following three cycles (median 0.024% or 0.0011 x 10^9/l, range 0.01-0.18% or .0008-0.014 x 10^9/l) and in 2/35 (5.7%) patients at the end of therapy (median 0.27% or 0.027 x 10^9/l, range 0.009-0.53% or 0.0004-0.053 x 10^9/l).

PFS was longer for patients without detectable WM B-cells in the BM compared to those with detectable WM B-cells following three cycles of therapy [3-year PFS: 100% (95%CI: 73.5-100) and 71.4% (95%CI: 47.2-86.0), respectively] as well as at the end of treatment [3-year PFS: 94.7% (95%CI: 68.1-99.2) and 63.2% (95%CI: 37.9-80.4), respectively]. In exploratory analyses, there was strong evidence that BM B-cell depletion after cycle three (HR=*not estimable*, p≤0.01) and at the end of treatment (HR=0.06, 95% CI: 0.01-0.47, p≤0.001) were associated with PFS (Figures 3A&B). There was no strong evidence of an association with baseline IPSSWM criteria (p=0.16), rIPSSWM criteria (p=0.29), or with end of treatment investigator-assessed response (p=0.16); furthermore, the observed association with BM B-cell depletion remained after adjusting for each of these, i.e. BM B-cell depletion is independently associated for PFS. Similar results were observed throughout for TTNT, although only 8 patients received further treatment for progression, there was evidence that BM B-cell depletion after cycle three (HR=*not estimable*, p=0.02) and at the end of treatment (HR=*not estimable*, p≤0.01) are independently associated with TTNT.

Toxicity of BRC

Six cycles of trial treatment were completed by 39 (92.9%) of the 42 patients who began BRC trial treatment. One patient was found to be ineligible and withdrawn

during cycle one, and two withdrew after four cycles due to toxicity (grade 2 oesophageal obstruction; grade 2 neuropathic pain). Dose reductions were needed for 16 (38.1%) patients, in 27 out of 243 total cycles: 21 due to adverse events (including 10 peripheral neuropathy and 6 neutropenia), 1 clinical decision, 4 were not treatment or disease related, and 1 reason was missing. Treatment delays occurred in 27 (64.3%) patients, in 46 out of 243 total cycles: 15 due to adverse events (including 6 neutropenia, 2 thrombocytopenia, 1 peripheral neuropathy and 1 renal insufficiency), 8 clinical or patient decision, 22 were not treatment or disease related, and 1 reason was missing.

Grade 3 or higher adverse events were reported in 27 (64.3%) BRC patients, with 19 (45.2%) being at least possibly-related to bortezomib. Only three (7.1%) patients had a grade 4 toxicity. No grade 3 or higher neuropathy was reported.

Discussion

In this multicentre, open-label, randomised phase II study of untreated patients with WM we show that the BRC combination is highly efficacious with an ORR of 97.6%, including a major response rate (MRR) of 80.5%, and 2-, 3- and 5-year PFS of 92.7%, 80.5% and 65.5%, respectively. We have demonstrated that WM B-cells may be depleted below the assay limit of detection (LoD) in a significant proportion of patients and that this has a profound impact on clinical outcome, in terms of reducing the risk of progression, independent of IgM response and IPSSWM/rIPSSWM status. We demonstrated for the first time prospectively that the absence of clonal B cells in the BM was associated with prolonged PFS and TTNT. With 45% of patients in R2W defined as having high risk disease, the outcomes achieved with BRC were

remarkable and exceeded our expectations given our target ORR of 80%. Alongside the excellent response, BRC was well tolerated with no problematic neuropathy-related adverse effects and limited haematological toxicity.

Based on these results, BRC can be considered as a front-line treatment option for patients with WM. Bendamustine plus rituximab (BR) and dexamethasone, rituximab and cyclophosphamide (DRC) are commonly used as primary therapy. BR is highly efficacious achieving a median PFS of 69.5 months in the StiL NHL1-2003 trial [15], however, a retrospective analysis of BR by the French Innovative Leukaemia Organization (FILO) suggests some caution is needed in the use of BR in this patient group.[16] 51% of patients experienced prolonged cytopenia, 44% needed dose reductions and we are mindful that this is a patient group who have a greater risk of developing secondary myeloid neoplasms. [17] The European prospective randomised study (ECWM-1) investigating bortezomib (B) in combination with dexamethasone, rituximab and cyclophosphamide (DRC) has recently shown similarly high levels of efficacy by combining bortezomib with standard immunochemotherapy.[18] With a median follow-up of 27.5 months, ORR was 91.2 % for B-DRC (MRR 79.1%) with a 2-year PFS of 80.6%. Although at this time point of analysis, adding Bortezomib to DRC did not induce significant differences in PFS compared to DRC alone, longer follow up is needed and a difference may emerge when medians have been reached. Despite the wide availability of BTK inhibitors, fixed duration immunochemotherapy remains the cornerstone of first-line treatment in WM and continues to be recommended in national and international guidelines. [1-3] BRC represents a highly efficacious, well-tolerated, fixed duration and cost-effective choice for patients in need of treatment.

Various groups have begun to investigate different residual disease monitoring techniques to establish a better indicator of outcome in WM rather than IgM response

In an early study using flow cytometry-based methodology, Garcia-Sanz and colleagues [19] showed a linear correlation between better quality of response and tumour B-cell counts in BM samples from 42 patients taken before and after treatment. B-cell depletion to <5% monoclonal B-cells correlated with better progression-free and overall survival. Molecular approaches are also possible utilising either the immunoglobulin heavy chain gene sequence, unique to each patient, or by using *MYD88 L265P*. The former has been developed in a range of B-cell disorders, most notably CLL and myeloma. Xu and colleagues [20] have demonstrated high sensitivity and specificity of an allele-specific real-time quantitative polymerase chain reaction (AS-qPCR) for *MYD88* and most recently Varettoni and colleagues [9] have applied similar methodology to assess impact of residual disease on outcomes in patients undergoing treatment. In a retrospective analysis of 54 patients from the FIL_BIOWM study they have demonstrated, for the first time, that molecular remission is an independent predictor of PFS. Droplet digital polymerase chain reaction (ddPCR) assays for *MYD88* have also been described [21,22] and may ultimately provide a reproducible platform for more widespread and routine use. They have been successfully used in a post-treatment pilot assessment, although no outcome data is yet available. [21] The ddPCR is, however, relatively insensitive when compared to flow cytometry (limit of detection 0.026-0.035% versus 0.0004% in our assay) but the method can be applied to cell-free DNA in peripheral blood where it appears to correlate with bone marrow burden suggesting a potential for non-invasive testing in the future.

The underlying cellular heterogeneity of WM should also be considered when assessing so-called minimal residual disease methodologies. In this study we have shown, at least in the context of rituximab-based therapy, that B-cell depletion is highly

predictive of outcome. This effect is best demonstrated with a WM-specific flow cytometry assay. It is possible that molecular based studies may not adequately demonstrate this effect as a residual molecular signal (*MYD88* or immunoglobulin sequence) may persist due to the residual plasma cell populations. These are typically long lived and explain slow IgM responses and the low incidence of CR with most conventional therapies. The current data do suggest that BM assessment, either by flow cytometry or molecular methods, provides for a better prediction of survival outcomes than conventional IgM response. Our data would also suggest that B-cell depletion should be the goal of therapy rather than serological complete response. It appears that the B-cell component is the main driver of the disease and that the plasma cells are merely secretory. In this context, BM B-cell depletion and / or molecular response could represent novel end-points for clinical trials as has been advocated in CLL and myeloma. This would be most relevant in the upfront setting where typical survival outcomes are long.

We conclude that BRC is a highly efficacious regimen for treatment-naïve WM patients having observed high response rates, durable outcomes and limited toxicity. Furthermore, we have shown, for the first time, that flow cytometric assessment of BM B-cell depletion is possible in WM and is independently associated with long-term outcomes. B-cell depletion was demonstrable following three cycles of treatment in the majority of patients suggesting that this assay could be used to evaluate treatment duration with the potential of limiting drug exposure in the future. Assessment of BM response should be considered in all future clinical studies particularly those assessing time-limited novel drug combinations.

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Author contributions

RdT was responsible for designing the laboratory flow cytometry assay, performing laboratory analyses, verifying and analysing data, interpreting results, making the figures and writing parts of the paper. NC was responsible for designing the trial, verifying and analysing data, interpreting results, making the figures and writing parts of the paper. L C-H was responsible for designing the trial and contributed to writing the paper. S D'S was responsible for designing the trial and performing the research by treating patients and collecting their data. GP was responsible for designing the trial and performing the research by treating patients and collecting their data. GC was responsible for performing the research by treating patients and collecting their data. LC was responsible for performing laboratory analyses. RS was responsible for performing laboratory analyses and analysing the data. WT was responsible for designing the trial and helped with writing of the trial protocol. BP was responsible for

designing the trial and helped with writing of the trial protocol. PS was responsible for designing the trial. OS was responsible for collecting the trial data. RO was responsible for responsible for designing the trial, performing the research by treating patients and collecting their data, interpreting results and writing parts of the paper. RA was responsible for designing the trial, performing the research by treating patients and collecting their data, interpreting results and writing the paper. As Chief Investigator of the trial she had overall responsibility for the trial and trial protocol.

All authors had full access to all the data in the study, critically reviewed the manuscript and approved the content. All authors accept responsibility to submit for publication.

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Figure 1. CONSORT diagram – registered patients (N=60).

Figure 2. Progression-Free Survival (2A), Time To Next Treatment (2B) and Overall Survival (2C) - BRC eligible patients who started trial treatment (N=41).

Figure 3. Progression-Free Survival by BM B-cell depletion after cycle 3 (3A) and at the end of treatment (3B) - BRC eligible patients who started trial treatment (N=41, where available).

Table 1. Baseline characteristics – BRC (N=43).

Baseline Characteristic	BCR (N=43)
Age in years, median (range)	67 (43,78)
Gender, n (%)	
Female	14 (33%)
Male	29 (67%)
IPSSWM risk group, n (%)	
Low	10 (23%)
Intermediate	15 (35%)
High	18 (42%)
Revised IPSSWM risk group, n (%)	
Very Low	5 (12%)
Low	10 (24%)
Intermediate	12 (29%)
High	11 (26%)
Very High	4 (10%)
ECOG performance status, n (%)	
0	18 (42%)
1	23 (53%)
2	2 (5%)
Lymphadenopathy present, n (%)	12 (28%)
Hepatomegaly present, n (%)	2 (5%)
Splenomegaly present, n (%)	7 (16%)
B Symptoms present, n (%)	9 (21%)
Peripheral neuropathy present, n (%)	8 (19%)
Signs of hyperviscosity present*, n (%)	28 (65%)
Plasma viscosity Cp, median (range), N=25	3.6 (1.9,8.5)
White blood cell $x10^9$/L, median (range)	6.2 (2.8,10.5)
Platelets $x10^9$/L, median (range)	200.0 (4.0,510.0)
Haemoglobin g/dL, median (range)	9.8 (6.5,14.0)
Absolute neutrophil count $x10^9$/L, median (range)	3.3 (1.4,7.2)
Lymphocytes $x10^9$/L, median (range)	1.7 (0.1,4.1)
Serum IgM paraprotein g/L, median (range)	32.0 (3.2,80.2)

*18 fatigue, 6 headache, 6 mucosal bleeding, 4 visual disturbance, 8 other (angina type chest pain; anginal symptoms; fainting; fullness/muzziness; light-headedness; neuropathy/epistaxis; odema; asymptomatic bilateral peripheral retinal haematology)

Table 2. Maximum grade 3-5 adverse events – BRC who started trial treatment (N=42).

Adverse Event	**BCR (N=42)**
Neutrophil count decreased	11* (26%)
Platelet count decreased	6** (14%)
Anaemia	5 (12%)
Diarrhoea	2 (5%)
Pain	2 (5%)
Back pain	1 (2%)
Febrile neutropenia	1 (2%)
Hearing impairment	1 (2%)
Heart failure	1 (2%)
Hypertension	1 (2%)
Hypotension	1 (2%)
Lung infection	1 (2%)
Neoplasms benign, malignant and unspecified	1 (2%)
Renal impairment	1 (2%)
Rituximab infusion reaction	1 (2%)
HIGHEST GRADE TOXICITY	
3	***24 (57%)***
4	***3 (7%)***
5	***0 (0%)***
ANY GRADE 3-5 TOXICITY	***27 (64%)***

**including 1x grade 4; **2x grade 4*