ORIGINAL ARTICLE

Ibrutinib in Previously Treated Waldenström's Macroglobulinemia

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ABSTRACT

BACKGROUND

MYD88^{L265P} and *CXCR4*^{WHIM} mutations are highly prevalent in Waldenström's macroglobulinemia. MYD88^{L265P} triggers tumor-cell growth through Bruton's tyrosine kinase, a target of ibrutinib. *CXCR4*^{WHIM} mutations confer in vitro resistance to ibrutinib.

METHODS

We performed a prospective study of ibrutinib in 63 symptomatic patients with Waldenström's macroglobulinemia who had received at least one previous treatment, and we investigated the effect of *MYD88* and *CXCR4* mutations on outcomes. Ibrutinib at a daily dose of 420 mg was administered orally until disease progression or the development of unacceptable toxic effects.

RESULTS

After the patients received ibrutinib, median serum IgM levels decreased from 3520 mg per deciliter to 880 mg per deciliter, median hemoglobin levels increased from 10.5 g per deciliter to 13.8 g per deciliter, and bone marrow involvement decreased from 60% to 25% (P<0.01 for all comparisons). The median time to at least a minor response was 4 weeks. The overall response rate was 90.5%, and the major response rate was 73.0%; these rates were highest among patients with *MYD88*^{L265P}*CXCR4*^{WT} (with WT indicating wild-type) (100% overall response rate and 91.2% major response rate), followed by patients with *MYD88*^{L265P}*CXCR4*^{WHIM} (85.7% and 61.9%, respectively) and patients with *MYD88*^{WT}*CXCR4*^{WT} (71.4% and 28.6%). The estimated 2-year progression-free and overall survival rates among all patients were 69.1% and 95.2%, respectively. Treatment-related toxic effects of grade 2 or higher included neutropenia (in 22% of the patients) and thrombocytopenia (in 14%), which were more common in heavily pretreated patients; postprocedural bleeding (in 3%); epistaxis associated with the use of fish-oil supplements (in 3%); and atrial fibrillation associated with a history of arrhythmia (5%).

CONCLUSIONS

Ibrutinib was highly active, associated with durable responses, and safe in pretreated patients with Waldenström's macroglobulinemia. *MYD88* and *CXCR4* mutation status affected responses to this drug. (Funded by Pharmacyclics and others; ClinicalTrials.gov number, NCT01614821.)

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Waldenström's macroglobulinemia is a malignant B-cell lymphoma that is associated with an accumulation of clonal lymphoplasmacytic cells and monoclonal IgM secretion.[1] Despite advances in treatment, the disease eventually progresses in most patients, and new treatment options are needed.

Whole-genome sequencing has revealed a single activating somatic mutation in *MYD88* (resulting in a predicted protein change from leucine to proline at amino acid position 265) and multiple activating mutations in the C-terminal domain of *CXCR4* in patients with Waldenström's macroglobulinemia.[2,3] In tumor cells, $MYD88^{L265P}$ triggers activation of nuclear factor κB (NF-κB) through two divergent pathways involving Bruton's tyrosine kinase (BTK) and the interleukin-1 receptor–associated kinases (IRAK1 and IRAK4).[4]

Ibrutinib is an orally administered, small-molecule inhibitor of BTK that triggers apoptosis of Waldenström's macroglobulinemia cells with $MYD88^{L265P}$. Clinical activity of ibrutinib in patients with Waldenström's macroglobulinemia was observed in a phase 1 study.[4,5]

Activating *CXCR4* somatic mutations in Waldenström's macroglobulinemia are similar to germline mutations detected in patients with the WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis).[6] At least 30 different $CXCR4^{WHIM}$ somatic mutations are present in Waldenström's macroglobulinemia.[3,7] Tumor cells engineered to express $CXCR4^{WHIM}$ receptors have been shown to have enhanced CXCL12-triggered activation of the prosurvival factors AKT and extracellular signal-regulated kinase (ERK) and decreased in vitro ibrutinib-related apoptosis.[8-10]

In Waldenström's macroglobulinemia, three genomic groups ($MYD88^{L265P}CXCR4^{WT}$ [with WT indicating wild-type], $MYD88^{L265P}CXCR4^{WHIM}$, and $MYD88^{WT}CXCR4^{WT}$) have been delineated on the basis of clinical manifestations and survival. These findings affirm an important role for *MYD88* and *CXCR4* somatic mutations in the pathogenesis of tumors.[7]

We performed a prospective, multicenter study of ibrutinib. We assessed safety and efficacy, as well as the influence of *MYD88* and *CXCR4* mutations on responses, in patients who had received previous treatment for Waldenström's macroglobulinemia.

METHODS

STUDY OVERSIGHT AND DESIGN

We conducted this study at three sites (the Dana–Farber Cancer Institute, Memorial Sloan Kettering Cancer Center, and Stanford University Medical Center). Enrollment began on May 23, 2012, and closed on June 13, 2013. The last patient evaluation and survival update occurred on December 19, 2014. All patients provided written informed consent after approval of the study by the institutional review boards at the three study sites.

Pharmacyclics and Janssen Pharmaceuticals supported this investigator-initiated study and provided research funding and the study drug. The first author designed the study, and all the authors vouch for the integrity of the data and adherence to the protocol (available with the full text of this article at NEJM.org). No one who is not an author contributed to the manuscript. The first author wrote all drafts of the manuscript. The academic authors administered the study drugs to the patients and collected the study data. The academic authors and Pharmacyclics coordinated the study, provided regulatory oversight, and analyzed the study data.

The primary objective was to determine the overall response rate, which included the rate of a minor response (≥25% reduction in serum IgM levels), partial response (≥50% reduction), very good partial response (≥90% reduction), and complete response, and to determine the rate of major response (a complete response or responses with a ≥50% reduction in serum IgM levels).[11] Secondary objectives included the determination of progression-free survival and drug safety.

Serum IgM and complete blood counts were obtained at the beginning of each cycle for 3 cycles and thereafter every 3 cycles. Bone marrow biopsies and computed tomography (CT) (if extramedullary disease was present at baseline) were repeated at cycles 6, 12, and 24, and annually thereafter. CT assessments were performed locally with the use of bidimensional (long-axis and short-axis) serial measurements of representative nodes.

PATIENTS

Eligibility criteria were the following: a need for treatment according to consensus guidelines, prior receipt of one or more treatment regimens, a platelet count of 50,000 per cubic millimeter or higher, a hemoglobin level of 8 g per deciliter or higher, an absolute neutrophil count of 1000 per cubic milli-

meter or higher, a serum creatinine level of 2 mg per deciliter or less, a total bilirubin level of 1.5 mg per deciliter or less (or <2 mg per deciliter if the level was attributable to the tumor), serum aspartate and alanine aminotransferase levels 2.5 times the upper limit of the normal range or less, and an Eastern Cooperative Oncology Group (ECOG) performance-status score of 2 or lower (on a scale of 0 to 5, with 0 indicating no symptoms and higher numbers indicating increasing tumor-related disability).[1,12] Patients were excluded if they had central nervous system lymphoma or clinically significant cardiovascular disease, if they were receiving warfarin, or if they were receiving medications that could prolong the QT interval.

The treatment regimen consisted of daily oral ibrutinib (at a dose of 420 mg) for twenty-six 4-week cycles until the disease progressed or unacceptable toxic effects developed. According to an amendment to the study protocol, patients without disease progression could provide a second informed consent and continue therapy beyond 26 cycles.

Ibrutinib was withheld if the following hematologic toxic effects occurred: a neutrophil count of less than 500 per cubic millimeter or a platelet count of less than 25,000 per cubic millimeter or less than 50,000 per cubic millimeter with bleeding. In addition, ibrutinib was withheld if the patient had nausea of grade 3 or higher, vomiting or diarrhea, or nonhematologic toxic effects of grade 3 or higher. Filgrastim therapy or transfusions were permitted. After ibrutinib was withheld the first time, full-dose retreatment was permitted after the patient recovered from toxic effects. Thereafter, reductions in the dose to 280 mg and then to 140 mg and, finally, discontinuation of the study drug were required as subsequent events occurred. In an amendment to the study protocol, it was recommended that to minimize the risk of bleeding, the drug should be withheld for 3 to 7 days before and for 1 to 3 days after an anticipated invasive procedure.

MYD88^{L265P} and *CXCR*^{WHIM} Mutation Genotyping

An allele-specific polymerase-chain-reaction (PCR) assay was used to detect *MYD88*^{L265P} mutations. *CXCR4*^{WHIM} mutation status was determined by means of Sanger sequencing, and allele-specific PCR was used to detect *CXCR4*^{S338X} C→G and C→A mutations in CD19-selected bone marrow cells.[3,7,13,14]

Statistical Analysis

A Simon's two-stage design was used with an alpha level set at 0.05 and a beta level set at 0.20; this assumed a null response rate of 20% and a successful overall response rate of 40% on the basis of comparisons with other monotherapies used in patients with previously treated Waldenström's macroglobulinemia. The protocol was amended according to regulatory guidance to require the enrollment of additional participants on the assumption that if the response rate for ibrutinib was 50%, the study would have more than 80% power to show a lower boundary of the two-sided 95% confidence interval for the response rate that would exceed 32%.

Responses were defined according to criteria adopted from the Third International Workshop on Waldenström's Macroglobulinemia.[11] Progression-free survival was defined as the time between the initiation of therapy and the date of disease progression, death, or last follow-up. The Kaplan–Meier method was used for time-to-event analyses with censoring. Pairwise comparisons were made with the use of a Wilcoxon rank-sum test with Bonferroni–Holm correction for multiple hypothesis testing. A Pearson correlation coefficient was used for linear comparisons. P values of 0.05 or less were considered to indicate statistical significance. Statistical analyses were performed with the use of SAS software, version 9.3 (SAS Institute).

RESULTS

Patients and Disease Characteristics

A total of 63 consecutive eligible patients (43 at the Dana–Farber Cancer Institute, 10 at Memorial Sloan Kettering Cancer Center, and 10 at Stanford University Medical Center) were enrolled. An independent, central pathology review confirmed that all the patients met the diagnostic criteria for Waldenström's macroglobulinemia.[1] The baseline characteristics of the patients are listed in Table 1.

MYD88^{L265P} tumor mutation status was determined in all 63 patients, and *CXCR4*^{WHIM} tumor mutation status was determined in 62 patients. *MYD88*^{L265P} was present in 56 patients (89%), and *CXCR4*^{WHIM} was present in 21 patients (34%). *CXCR4*^{WHIM} mutations included nonsense mutations (16 *CXCR4*^{S338X} and 2 *CXCR4*^{R334X}) and frameshift mutations (1 S324fs and 2 S338fs). All patients with wild-type *MYD88* had wild-type *CXCR4*, a finding consistent with prior findings.[3,7] No significant baseline differences according to *MYD88* or

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Patients (N = 63)
Median age (range) — yr	63 (44–86)
Sex — no.	
Male	48
Female	15
Median time from diagnosis of Waldenström's macroglobulinemia (range) — mo	76 (6–340)
Previous therapy for Waldenström's macroglobulinemia	
Median no. of previous treatment regimens (range)	2 (1–9)
Type of therapy — no. (%)	
Monoclonal antibody	57 (90)
Glucocorticoid	42 (67)
Proteasome inhibitor	33 (52)
Alkylator	32 (51)
Nucleoside analogue	15 (24)
MTOR inhibitor	13 (21)
Immunomodulator	7 (11)
Anthracycline	7 (11)
Autologous transplantation	4 (6)
Other, including experimental therapy	13 (21)
Disease refractory to most recent regimen	25 (40)
Waldenström's macroglobulinemia IPSS score — no. (%)†	
Low	14 (22)
Intermediate	27 (43)
High	22 (35)
Serum antibody levels	
IgM	
Median (range) — mg/dl	3520 (724–8390)
>4000 mg/dl — no. (%)	26 (41)
Median IgA (range) — mg/dl	26 (0–125)
Median IgG (range) — mg/dl	381 (49–2770)
Median absolute neutrophil count (range) — per mm^3	3.18 (1.14–10.97)
Hemoglobin level	
Median (range) — g/dl	10.5 (8.2–13.8)
<11 g/dl — no. (%)	37 (59)
<10 g/dl — no. (%)	25 (40)
Median hematocrit (range) — (%)	30.8 (24.5–41.5)
Platelet count	
Median (range) — per mm^3	214,000 (24,000–459,000)
<100,000/mm^3 — no. (%)	7 (11)
Serum β_2-microglobulin	
Median (range) — mg/liter	3.9 (1.3–14.2)
>3 mg/liter — no. (%)	45 (71)
>3.5 mg/liter — no. (%)	35 (56)
Adenopathy ≥1.5 cm — no. (%)	37 (59)
Splenomegaly ≥15 cm — no. (%)	7 (11)
Median bone marrow involvement (range) — %	60 (3–95)

* IPSS denotes International Prognostic Scoring System, and mTOR mammalian target of rapamycin.
† The Waldenström's macroglobulinemia International Prognostic Scoring System (IPSS) to assess the risk of death is based on five adverse covariates: advanced age (>65 years), a hemoglobin level of 11.5 g per deciliter or more, a platelet count of 100,000 per cubic millimeter or less, a β_2-microglobulin level higher than 3 mg per liter, and a serum monoclonal protein concentration higher than 7.0 g per deciliter. Low-risk patients do not have advanced age and have either no adverse covariates or one adverse covariate, intermediate-risk patients have two adverse covariates or only advanced age, and high-risk patients have more than two adverse covariates.

CXCR4 mutation status were observed, except for a higher incidence of adenopathy among patients with wild-type *CXCR4* than among those with $CXCR4^{WHIM}$ (68.3% vs. 33.3%, P=0.01), a finding consistent with previous observations.[7] The median duration of treatment was 19.1 months (range, 0.5 to 29.7).

RESPONSES

The median serum IgM level decreased from 3520 mg per deciliter to 880 mg per deciliter at the time of best response in the 63 patients overall (P<0.001). Before therapy, 46 of the 63 patients (73%) had a serum IgM level of 3000 mg per deciliter or higher; after treatment, at the time of best response, 6 of the 63 patients (10%) had a serum IgM level of 3000 mg per deciliter or higher (P<0.001).

Median bone marrow involvement decreased from 60% to 25% (P<0.001), and the median hemoglobin level increased from 10.5 g per deciliter to 13.8 g per deciliter at the time of best response (P<0.001). Discordance between serum IgM levels and bone marrow involvement was observed at 6 months (r=0.03, P=0.83), though by 12 months (r=0.51, P<0.001) and 24 months (r=0.56, P<0.008), a stronger correlation of these variables was evident.

Responses included a very good partial response in 10 patients, a partial response in 36 patients, and a minor response in 11 patients, representing overall and major responses of 90.5% (95% confidence interval [CI], 80.4 to 96.4) and 73.0% (95% CI, 60.3 to 83.4), respectively. The median times to at least minor and partial responses were 4 weeks and 8 weeks, respectively.

The rate of overall response was similar regardless of baseline age (<65 vs. ≥65 years), ECOG performance-status score (0 vs. ≥1), pretherapy International Prognostic Scoring System (IPSS) score for patients with Waldenström's macroglobulinemia (see Table S1 in the Supplementary Appendix, available at NEJM.org),[15] serum β_2-microglobulin level (≤3 vs. >3 mg per liter), hemoglobin level (<11 vs. ≥11 g per deciliter), serum IgM level (<4000 vs. ≥4000 mg per deciliter), bone marrow involvement (<50% vs. ≥ 50%), prior relapsed or refractory disease, and the number of previous treatment regimens (1 to 3 vs. >3). The rate of major response was also similar across most of the baseline subgroups (Fig. 1A and 1B), and rate of response was similar across the three participating institutions.

Overall and major response rates were highest among patients with the $MYD88^{L265P}CXCR4^{WT}$ genotype (100.0% and 91.2%, respectively), followed by $MYD88^{L265P}CXCR4^{WHIM}$ (85.7% and 61.9%) and $MYD88^{WT}CXCR4^{WT}$ (71.4% and 28.6%) (Fig. 1A and 1B). Improvements in overall and major response rates occurred in all three genomic subgroups with prolonged therapy (>6 cycles) but were more pronounced among patients with $MYD88^{L265P}CXCR4^{WHIM}$ mutations (Fig S1 in the Supplementary Appendix). Best serum IgM (Fig. 2A) and hemoglobin (Fig. 2B) responses were also influenced by tumor genotype; improvements were most evident in patients with $MYD88^{L265P}CXCR4^{WT}$ and least evident in those with $MYD88^{WT}CXCR4^{WT}$.

CT-identified adenopathy (≥1.5 cm) was present in 37 patients at baseline. Serial imaging in 35 patients showed decreased or resolved adenopathy in 25 patients (68%), stable adenopathy in 9 patients (24%), and increased adenopathy in 1 patient (3%). Two patients discontinued the study before repeat imaging was required.

Among 7 patients with CT-identified splenomegaly (≥15 cm), spleen size was decreased in 4 patients (57%), stable in 2 patients (29%), and could not be evaluated in 1 patient (14%) after elective splenectomy. Nine patients (14%), 3 of whom had anti–myelin-associated glycoprotein antibodies, received ibrutinib for progressive IgM-related peripheral sensory neuropathy. All 9 patients had a response, and subjective improvements in peripheral sensory neuropathy occurred in 5 patients and remained stable in 4 patients during the treatment course.

Symptomatic hyperviscosity related to progressive disease that necessitated plasmapheresis prompted the initiation of ibrutinib in 4 patients. All had a response, and none required additional plasmapheresis by the end of cycle 2. One patient required plasmapheresis for acquired factor VIII deficiency. He had a response and did not require further plasmapheresis. The spontaneous bleeding events that prompted therapy also resolved, and he continued to receive ibrutinib.

TREATMENT PERIOD

The median duration of treatment was 19.1 months (range, 0.5 to 29.7); 43 patients (68%) continued to receive therapy after the database was locked (on December 19, 2014). Reasons for discontinuation of treatment included nonresponse (1 patient), progressive disease (7 patients), treatment-aggravated thrombocytopenia (1 pa-

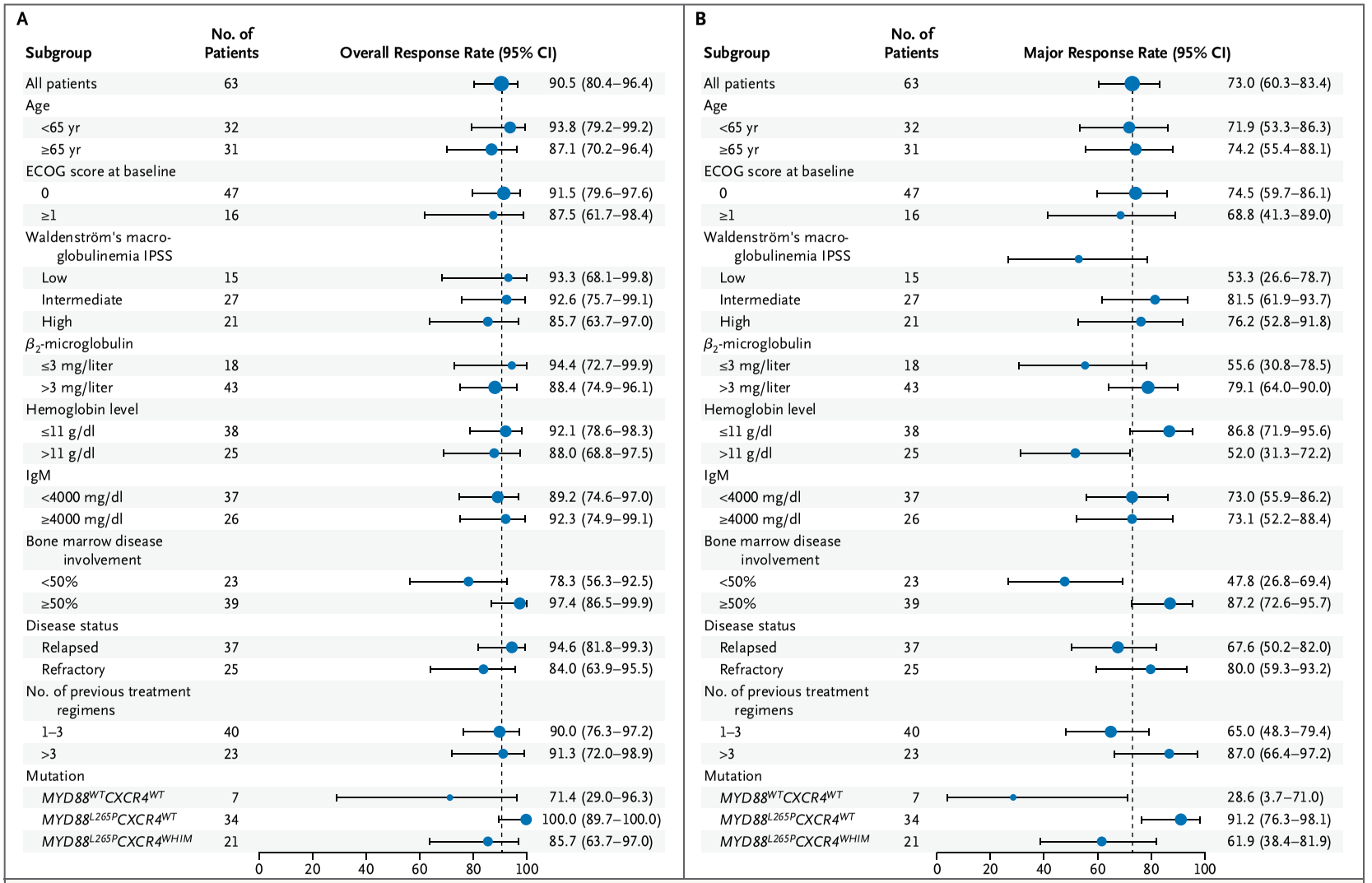

Figure 1. Subgroup Analyses of Responses.

Subgroup analyses of overall responses (Panel A) and major responses (Panel B) are shown. Eastern Cooperative Oncology Group (ECOG) performance-status scores range from 0 to 5, with 0 indicating no symptoms and higher numbers indicating increasing tumor-related disability. The Waldenström's macroglobulinemia International Prognostic Scoring System (IPSS) to assess the risk of death is based on five adverse covariates: advanced age (>65 years), a hemoglobin level of 11.5 g per deciliter or more, a platelet count of 100,000 per cubic millimeter or less, a β_2-microglobulin level higher than 3 mg per liter, and a serum monoclonal protein concentration higher than 7.0 g per deciliter. Low-risk patients do not have advanced age and have either no adverse covariates or one adverse covariate, intermediate-risk patients have two adverse covariates or only advanced age, and high-risk patients have more than two adverse covariates. Bone marrow disease involvement was determined by means of direct morphologic assessment after hematoxylin and eosin staining of a histopathological section and enumeration of lymphoplasmacytic cells occupying the intertrabecular space on complete representative slides.

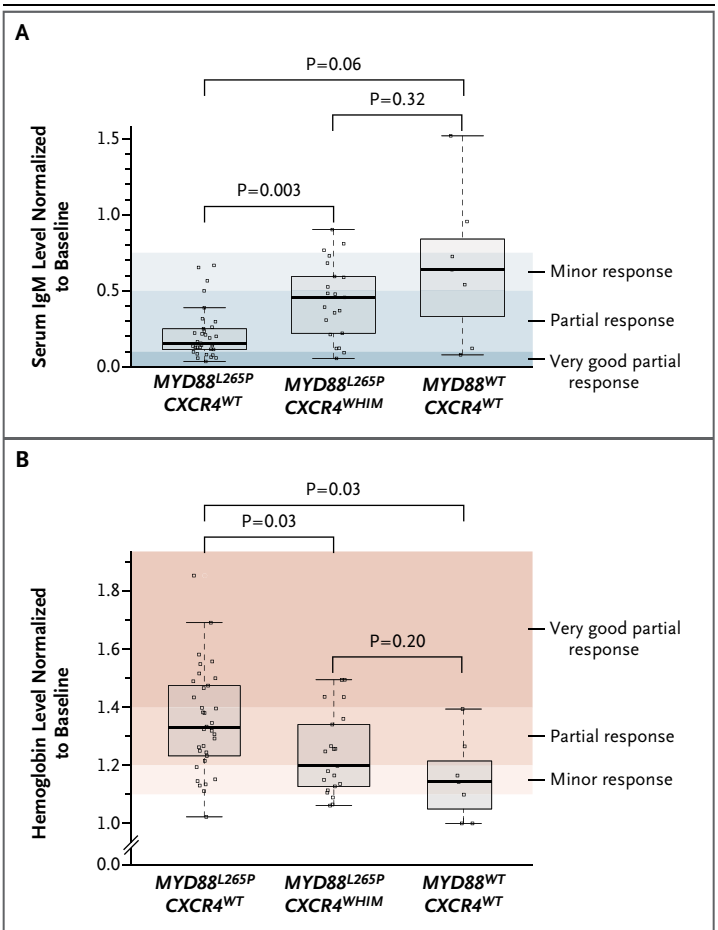

Figure 2. Effect of *MYD88* and *CXCR4* Mutation Status on Ibrutinib-Related Changes in Serum IgM and Hemoglobin Levels.

Serum IgM levels (Panel A) and hemoglobin levels (Panel B) at the time of best response in the 63 patients with Waldenström's macroglobulinemia were stratified according to *MYD88* and *CXCR4* mutation status. The thick horizontal lines represent the median values, and the lower and upper boundaries of the boxes the interquartile ranges. I bars encompass data points that are within 1.5 times the interquartile range from the first or third quartile.

tient), hematoma after bone marrow biopsy (1 patient), prolonged withholding of the drug because of infection unrelated to ibrutinib (1 patient), myelodysplasia and acute myeloid leukemia associated with baseline 5q deletion related to prior therapies (1 patient), disease transformation possibly related to prior nucleoside analogue therapy (2 patients), antineoplastic therapy for rectal carcinoma (1 patient), ibrutinib-incompatible medication (1 patient), the patient's decision to use commercially sourced ibrutinib (2 patients), travel difficulties (1 patient), and alternative therapy (1 patient).

PROGRESSION-FREE AND OVERALL SURVIVAL

Of the 63 patients enrolled, 60 patients were alive on the date on which the database was locked. Kaplan–Meier curves for progression-free survival among all 63 patients are shown in Figure 3A and for overall survival in Figure 3B. At 24 months, the estimated rate of progression-free survival was 69.1% (95% CI, 53.2 to 80.5), and the estimated rate of overall survival was 95.2% (95% CI, 86.0 to 98.4). Among patients with progressive disease, the median time to progression was 9.6 months (range, 3.5 to 19.4) if data on transformation events were censored, and 9.5 months (range, 3.5 to 19.4) if data on transformation events were included. Subgroup analysis showed that a high IPSS score before therapy, more than three previous treatment regimens, and a *MYD88*^{WT} *CXCR4*^{WT} genotype were associated with lower rates of progression-free survival (Fig. S2 in the Supplementary Appendix).

TOXIC EFFECTS

Grade 2 or higher treatment-related toxic effects are listed in Table 2. Neutropenia of grade 3 or higher occurred in 9 patients (14%), 7 of whom (78%) had received three or more prior therapies (P=0.05). Thrombocytopenia of grade 3 or higher occurred in 8 patients (13%), 7 of whom (88%) had received three or more prior therapies (P=0.01). Ibrutinib-related neutropenia and thrombocytopenia were reversible, although they necessitated dose reduction in 3 patients and treatment discontinuation in 4 patients.

Grade 2 or higher bleeding events occurred in 4 patients (2 of whom had epistaxis and 2 of whom had postprocedural bleeding before the study was amended to mandate withholding times for ibrutinib). Fish-oil supplements contributed to both grade 2 epistaxis events, and these events resolved when these supplements were discontinued.

Infections that were at least possibly associated with ibrutinib were few, and in most cases, patients with infections had preexisting IgA and IgG hypogammaglobulinemia. One patient with IgA and IgG hypogammaglobulinemia had streptococcal bacteremia and uncomplicated endocarditis after a dental procedure.

Atrial fibrillation related to ibrutinib occurred in 3 patients, all of whom had a history of paroxysmal atrial fibrillation. Atrial fibrillation resolved after ibrutinib was withheld, without cardiologic intervention, and protocol therapy resumed uneventfully in all 3 patients. Dose reductions due to toxic

effects, which occurred in 10 patients, did not influence responses or progression-free survival.

EFFECT OF IBRUTINIB ON PERIPHERAL LYMPHOCYTOSIS

Since transduction through the CXCR4 receptor promotes bone marrow homing of Waldenström's macroglobulinemia cells,[16] we next examined the influence of *MYD88* and *CXCR4* mutation status on ibrutinib-related peripheral lymphocytosis. This influence was previously observed in chronic lymphoid leukemia and mantle-cell lymphoma.[17-20]

At baseline, patients with $MYD88^{L265P}CXCR4^{WT}$, $MYD88^{L265P}CXCR4^{WHIM}$, and $MYD88^{WT}CXCR4^{WT}$ had similar levels of low circulating lymphocytes (P=0.74 for the comparison of $MYD88^{L265P}CXCR4^{WT}$ vs. $MYD88^{L265P}CXCR4^{WHIM}$, P=0.79 for the comparison of $MYD88^{L265P}CXCR4^{WT}$ vs. $MYD88^{WT}CXCR4^{WT}$, and P=0.92 for the comparison of $MYD88^{L265P}CXCR4^{WHIM}$ vs. $MYD88^{WT}CXCR4^{WT}$). Increases in median absolute lymphocyte levels were more pronounced within the first 6 months after the initiation of ibrutinib therapy in patients with $MYD88^{L265P}CXCR4^{WT}$ than in patients with $MYD88^{L265P}CXCR4^{WHIM}$ (P=0.04).

Patients with $MYD88^{WT}CXCR4^{WT}$ who typically have peripheral lymphocytosis had intermediate levels of increased peripheral lymphocytosis as compared with patients with $MYD88^{L265P}CXCR4^{WT}$ and $MYD88^{L265P}CXCR4^{WHIM}$ (Fig. S3A in the Supplementary Appendix) (P not significant). Higher levels of peripheral lymphocytosis were also observed in patients who had major (partial or very good partial) responses (Fig. S3B in the Supplementary Appendix) (P=0.01).

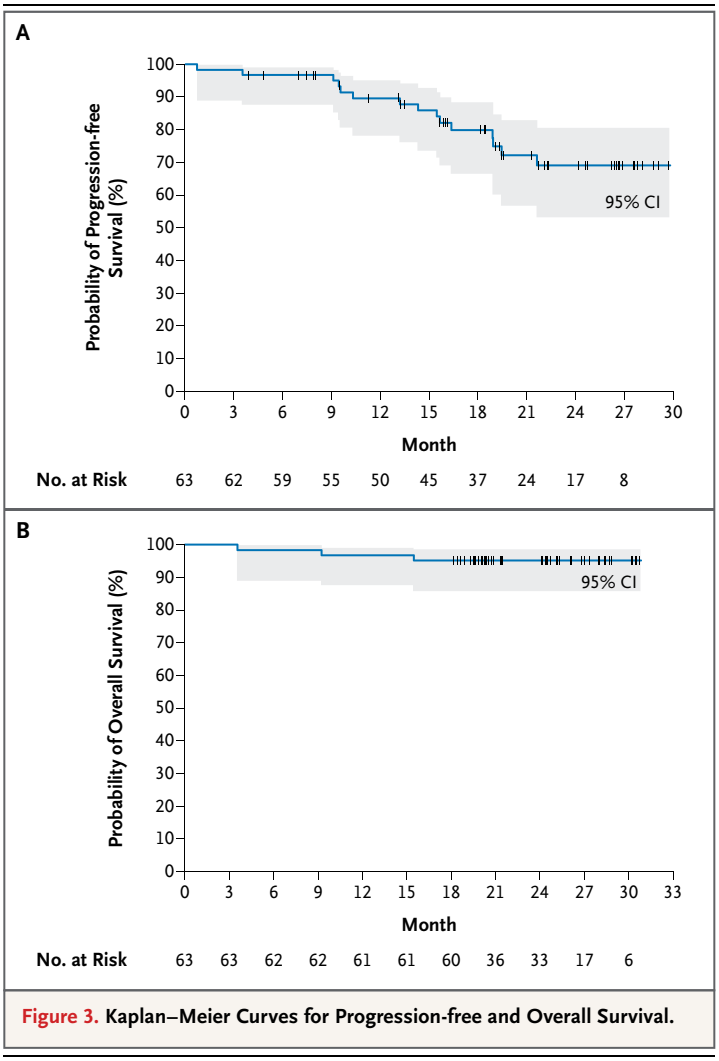

Figure 3. Kaplan–Meier Curves for Progression-free and Overall Survival.

DISCUSSION

The high prevalence of $MYD88^{L265P}$ in Waldenström's macroglobulinemia and its influence on tumor-cell survival through BTK-triggered NF-κB activation prompted us to perform this prospective, multicenter study of ibrutinib in previously treated patients with Waldenström's macroglobulinemia.[2-4,21,22] A high overall response rate (90.5%) and major response rate (73.0%) were observed among participants who had received a median of two prior therapies, and 40% of these patients had disease that was refractory to previous therapy. Moreover, at 2 years, the progression-free survival rate was 69.1% and the overall survival rate was 95.2%. By comparison, response rates of 40 to 80% with a median progression-free survival of 8 to 20 months have been reported with other monotherapies in patients with relapsed or refractory Waldenström's macroglobulinemia.[23,24]

Ibrutinib, as compared with most other available therapies, also showed rapid response kinetics, with a median time to response of 4 weeks. Among patients who had a response, the median hemoglobin level increased from 10.3 g per deciliter at baseline to 11.4 g per deciliter at 4 weeks and 12.0 g per deciliter at 8 weeks. Improvements in serum IgM and hemoglobin levels occurred even in patients with modest or no changes in bone marrow disease burden; this suggests that a mechanism other than tumor debulking could contribute to the clinical benefit with ibrutinib in patients with Waldenström's macroglobulinemia.

Discordance between serum IgM levels and marrow disease burden has been reported with other therapeutic agents in Waldenström's macroglobulinemia.[25-28] The BTK substrate STAT5A regu-

Table 2. Adverse Events Associated with Ibrutinib Therapy.*

Event or Abnormality	Grade 2	Grade 3	Grade 4	Total Grades 2–4
	number of patients (percent)			
Blood and lymphatic system disorders				
Neutropenia	5 (8)	6 (10)	3 (5)	14 (22)
Thrombocytopenia	1 (2)	6 (10)	2 (3)	9 (14)
Anemia	3 (5)	1 (2)	0	4 (6)
Febrile neutropenia	0	0	1 (2)	1 (2)
Cardiac disorders				
Atrial fibrillation	2 (3)	1 (2)	0	3 (5)
Sinus tachycardia	1 (2)	0	0	1 (2)
Gastrointestinal disorders				
Gastroesophageal reflux	3 (5)	0	0	3 (5)
Stomatitis	3 (5)	0	0	3 (5)
Constipation	2 (3)	0	0	2 (3)
Diarrhea	2 (3)	0	0	2 (3)
Ulceration	2 (3)	0	0	2 (3)
Infections and infestations				
Pneumonia	4 (6)	1 (2)	0	5 (8)
Skin infection	3 (5)	0	0	3 (5)
Cellulitis	1 (2)	0	0	1 (2)
Herpes zoster	1 (2)	1 (2)	0	2 (3)
Sinusitis	1 (2)	0	0	1 (2)
Streptococcal endocarditis	0	1 (2)	0	1 (2)
Subcutaneous abscess	1 (2)	1 (2)	0	1 (2)
Urinary tract infection	1 (2)	1 (2)	0	1 (2)
Postprocedural complications				
Hematoma	0	1 (2)	0	1 (2)
Hemorrhage	1 (2)	0	0	1 (2)
Dehydration	2 (3)	0	0	2 (3)
Musculoskeletal and connective-tissue disorders				
Tendinitis	1 (2)	0	0	1 (2)
Tenosynovitis	1 (2)	0	0	1 (2)
Nervous system disorders				
Headache	1 (2)	0	0	1 (2)
Presyncope	1 (2)	0	0	1 (2)
Syncope	0	1 (2)	0	1 (2)
Respiratory, thoracic, and mediastinal disorders				
Epistaxis	2 (3)	0	0	2 (3)
Cough	1 (2)	0	0	1 (2)
Skin and subcutaneous tissue disorders				
Pruritus	1 (2)	0	0	1 (2)
Rash	1 (2)	0	0	1 (2)
Skin exfoliation	1 (2)	0	0	1 (2)
Vascular disorders				
Hypertension	3 (5)	0	0	3 (5)
Hypotension	1 (2)	0	0	1 (2)

* Listed are adverse events that were deemed by the investigators to be possibly, probably, or definitely associated with the study drug.

lates IgM secretion in these tumor cells, and its selective inhibition by ibrutinib may have contributed to the early discordant findings.[29,30] Transient increases in serum IgM levels commonly occurred during periods in which the drug was withheld because of toxic effects or procedures, and these levels decreased with reinstitution of therapy.

Extramedullary disease was also affected by ibrutinib therapy; 68% of the patients had decreased or resolved adenopathy, and 57% had decreased splenomegaly on serial CT imaging. Resolution of malignant pleural effusions also occurred in 2 of 3 patients. Progressive IgM-related peripheral neuropathy prompted therapy in 9 patients who had previously received rituximab. Subjective improvement or stabilization of symptoms occurred during the course of treatment in these patients. These findings are particularly encouraging given the challenging nature of treating IgM-related peripheral neuropathy in Waldenström's macroglobulinemia.[23,31] Up to 25% of patients with Waldenström's macroglobulinemia have paraprotein-related peripheral neuropathy, and rituximab remains a mainstay of treatment. A recent placebo-controlled study showed no objective evidence of symptomatic improvement with the use of rituximab in patients with IgM-related peripheral neuropathy.[32] Moreover, rituximab can often cause a flare in serum IgM levels that can potentiate symptoms of peripheral neuropathy.[23,24] In our study, no IgM flare was observed in any of the 63 study patients who received ibrutinib.

Overall and major response rates were highest among patients with $MYD88^{L265P}CXCR4^{WT}$ (100% and 91.2%, respectively), followed by those with $MYD88^{L265P}CXCR4^{WHIM}$ (85.7% and 61.9%), and those with $MYD88^{WT}CXCR4^{WT}$ (71.4% and 28.6%). Although these findings are based on a small cohort of patients with Waldenström's macroglobulinemia, they probably reflect BTK dependence on $MYD88^{L265P}$-triggered signaling and intrinsic resistance conferred by $CXCR4^{WHIM}$ mutations in Waldenström's macroglobulinemia cells.[2,4,8-10] Furthermore, bone marrow homing of Waldenström's macroglobulinemia cells engineered to express the $CXCR4^{S338X}$ receptor could be inhibited by a CXCR4-blocking antibody; this is a relevant finding, since the bone marrow stroma protects the tumor cells against many therapeutic agents.[10] These insights may also help to explain why the incidence of ibrutinib-triggered peripheral lymphocytosis was higher among patients with $MYD88^{L265P}CXCR4^{WT}$ than among patients with $MYD88^{L265P}CXCR4^{WHIM}$. They may also account for the better categorical responses in patients with more pronounced peripheral lymphocytosis.

These findings are also likely to herald efforts to combine CXCR4 antagonists with ibrutinib in patients with $CXCR4^{WHIM}$ mutations. Plerixafor, a CXCR4 antagonist approved by the Food and Drug Administration for use in stem-cell mobilization, sensitizes Waldenström's macroglobulinemia cells engineered to express $CXCR4^{WHIM}$ receptors to undergo apoptosis in response to ibrutinib.[8,9] The feasibility of long-term use of plerixafor has been reported in patients with the WHIM syndrome, and clinical trials of other CXCR4 inhibitors are ongoing.[33]

Improvements in overall and major responses occurred in all three genomic subgroups with prolonged therapy (>6 cycles) but were more pronounced in patients with $MYD88^{L265P}CXCR4^{WHIM}$ mutations. A short course of CXCR4 inhibition during the initial phase of ibrutinib therapy may therefore be sufficient to prompt earlier and deeper responses to ibrutinib in patients with Waldenström's macroglobulinemia who have *CXCR4* mutations.

Overall, toxic effects of treatment were moderate in this selected patient population. Heavily pretreated patients were more at risk for clinically significant neutropenia and thrombocytopenia than were patients with fewer previous treatment regimens. Four bleeding events were related to procedures or fish-oil supplements. Atrial fibrillation related to ibrutinib occurred in 3 patients (5%), all of whom had a history of arrhythmia, and atrial fibrillation reverted after ibrutinib was withheld. In a randomized study, atrial fibrillation was observed in 3% of patients with chronic lymphoid leukemia who received ibrutinib, as compared with no patients who received ofatumumab.[34] Few infections were associated with ibrutinib, and this drug did not significantly alter serum IgA and IgG levels (as was also observed in patients with chronic lymphoid leukemia and patients with mantle-cell lymphoma).[17,18] Collectively, safety, along with the IgA-sparing and IgG-sparing effects, distinguish ibrutinib from other salvage options in Waldenström's macroglobulinemia.[23,24]

In summary, ibrutinib was active in previously treated patients with Waldenström's macroglobulinemia. An overall response rate of 90.5%, and 2-year progression-free and overall survival rates of 69.1% and 95.2%, respectively, were achieved with

ibrutinib monotherapy. Responses to ibrutinib were influenced by *MYD88* and *CXCR4* mutation status. Overall, toxic effects of treatment were moderate, and no unexpected toxic effects were observed.

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REFERENCES

1. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:110-5.

2. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. N Engl J Med 2012; 367:826-33.

3. Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood 2014;123:1637-46.

4. Yang G, Zhou Y, Liu X, et al. A mutation in MYD88 (L265P) supports the survival of lymphoplasmacytic cells by activation of Bruton tyrosine kinase in Waldenström macroglobulinemia. Blood 2013;122:1222-32.

5. Advani RH, Buggy JJ, Sharman JP, et al. Bruton tyrosine kinase inhibitor ibrutinib (PCI-32765) has significant activity in patients with relapsed/refractory B-cell malignancies. J Clin Oncol 2013;31:88-94.

6. Dotta L, Tassone L, Badolato R. Clinical and genetic features of warts, hypogammaglobulinemia, infections and myelokathexis (WHIM) syndrome. Curr Mol Med 2011;11: 317-25.

7. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood 2014; 123:2791-6.

8. Cao Y, Hunter ZR, Liu X, et al. The WHIM-like CXCR4S338X somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenstrom's macroglobulinemia. Leukemia 2015;29:169-76.

9. Cao Y, Hunter ZR, Liu X, et al. CXCR4 WHIM-like frameshift and nonsense mutations promote ibrutinib resistance but do not supplant MYD88L265P-directed survival signaling in Waldenstrom macroglobulinemia cells. Br J Haematol 2015; 168:701-7.

10. Roccaro AM, Sacco A, Jimenez C, et al. C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. Blood 2014;123:4120-31.

11. Anderson KC, Alsina M, Bensinger W, et al. Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma, version 2.2013. J Natl Compr Canc Netw 2012;10: 1211-9.

12. Kyle RA, Treon SP, Alexanian R, et al. Prognostic markers and criteria to initiate therapy in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:116-20.

13. Xu L, Hunter ZR, Yang G, et al. MYD88 L265P in Waldenström macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. Blood 2013; 121:2051-8.

14. Xu L, Hunter ZR, Yang G, et al. Detection of MYD88 L265P in peripheral blood of patients with Waldenström's macroglobulinemia and IgM monoclonal gammopathy of undetermined significance. Leukemia 2014;28:1698-704.

15. Morel P, Duhamel A, Gobbi P, et al. International prognostic scoring system for Waldenstrom macroglobulinemia. Blood 2009;113:4163-70.

16. Ngo HT, Leleu X, Lee J, et al. SDF-1/CXCR4 and VLA-4 interaction regulates homing in Waldenstrom macroglobulinemia. Blood 2008;112:150-8.

17. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med 2013;369:32-42.

18. Wang ML, Rule S, Martin P, et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med 2013;369:507-16.

19. de Rooij MF, Kuil A, Geest CR, et al. The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia. Blood 2012;119:2590-4.

20. Herman SE, Niemann CU, Farooqui M, et al. Ibrutinib-induced lymphocytosis in patients with chronic lymphocytic leukemia: correlative analyses from a phase II study. Leukemia 2014;28:2188-96.

21. Jiménez C, Sebastián E, Chillón MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenström's macroglobulinemia. Leukemia 2013;27:1722-8.

22. Poulain S, Roumier C, Decambron A, et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood 2013; 121:4504-11.

23. Treon SP. How I treat Waldenström macroglobulinemia. Blood 2009;114:2375-85.

24. Dimopoulos M, Kastritis E, Owen R, et al. Treatment recommendations for patients with Waldenström's macroglobulinemia (WM) and related disorders: IWWM-7 consensus. Blood 2014;124:1404-11.

25. Dimopoulos MA, Zervas C, Zomas A, et al. Treatment of Waldenström's macroglobulinemia with rituximab. J Clin Oncol 2002; 20:2327-33.

26. Chen CI, Kouroukis CT, White D, et al. Bortezomib is active in patients with untreated or relapsed Waldenstrom's macroglobulinemia: a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007;25:1570-5.

27. Strauss SJ, Maharaj L, Hoare S, et al. Bortezomib therapy in patients with relapsed or refractory lymphoma: potential correlation of in vitro sensitivity and tumor necrosis factor alpha response with clinical activity. J Clin Oncol 2006;24:2105-12.

28. Treon SP, Tripsas CK, Ioakimidis L, et al. Prospective, multicenter study of the MTOR-inhibitor everolimus (RAD001) as primary therapy in Waldenstrom's macroglobulinemia. Presented at the 53rd ASH Annual Meeting and Exposition, San Diego, December 10–13, 2011. abstract (https://ash.confex.com/ash/2011/webprogram/Paper44858.html).

29. Hodge LS, Ziesmer SC, Yang ZZ, Secreto FJ, Novak AJ, Ansell SM. Constitutive activation of STAT5A and STAT5B regulates IgM secretion in Waldenstrom's macroglobulinemia. Blood 2014;123:1055-8.

30. Mahajan S, Vassilev A, Sun N, Ozer Z, Mao C, Uckun FM. Transcription factor STAT5A is a substrate of Bruton's tyrosine kinase in B cells. J Biol Chem 2001;276: 31216-28.

31. Lunn MP, Nobile-Orazio E. Immunotherapy for IgM anti-myelin-associated glycoprotein paraprotein-associated peripheral neuropathies. Cochrane Database Syst Rev 2012;5:CD002827.

32. Léger JM, Viala K, Nicolas G, et al. Placebo-controlled trial of rituximab in IgM anti-myelin-associated glycoprotein neuropathy. Neurology 2013;80:2217-25.

33. McDermott DH, Liu Q, Ulrick J, et al. The CXCR4 antagonist plerixafor corrects panleukopenia in patients with WHIM syndrome. Blood 2011;118:4957-62.

34. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med 2014;371:213-23.